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(57) Abstract

The present invention relates generally to novel molecules and more particularly novel proteinaceous molecules involved in or associated with regulation of cell activities and/or viability. The present invention is particularly directed to novel serine proteinases and a novel kinase and to derivatives, agonists and antagonists thereof. In one embodiment, the present invention provides a novel serine proteinase, referred to herein as "HELA2" or "testisin", which has roles in spermatogenesis, in suppressing testicular cancer and as a marker for cancers.

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NOVEL MOLECULES

FIELD OF THE INVENTION

The present invention related generally to novel molecules and more particularly novel proteinaceous molecules involved in or associated with regulation of cell activities and/or viability. The present invention is particularly directed to novel serine proteinases and a novel kinase and to derivatives, agonists and antagonists thereof. In one embodiment, the present invention provides a novel serine proteinase, referred to herein as "HELA2" or "testisin", which has roles in spermatogenesis, in suppressing testicular cancer and as a marker for cancers.

BACKGROUND OF THE INVENTION

The rapidly increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical and allied health fields. This is particularly the case in the area of cell regulation leading to a greater understanding of the events leading to or involved in cancer, development of acquired immunodeficiency disease syndrome (AIDS), neurological disorders, heart disease, tissue graft rejection and infertility amongst many other conditions.

Two particularly important classes of molecules are the proteinases and kinases.

Proteinases play important roles in a number of physiological and pathological processes such as proteolytic cascades involved in blood coagulation, fibrinolysis and complement activation as well as cleavage of growth factors, hormones and receptors, the release of bioactive molecules and processes involving cell proliferation and development, inflammation, tumour growth and metastasis. Of particular significance are the cellular proteinases, or those proteinases synthesized in cells and tissues which serve to activate or deactivate proteins responsible for performing specific functions. These proteinases may be found outside the cell, within the cell or may be present on the cell surface.

Serine proteinases are particularly important. These proteinases are characterised by a

mechanism involving serine, histidine and aspartate amino acids in the serine proteinase active site. Members of the serine proteinase family which play important roles in a range of cellular functions and which have demonstrated causative roles in human diseases include tissue-type plasminogen activator and thrombin (thrombosis and blood clotting), urokinase-type plasminogen activator (cancer and metastasis), trypsin and elastase (emphysema and liver disease) and angiotensin converting enzyme (hypertension).

A serine proteinase is also implicated in TNFα degradation and soluble TNF-receptor (p75) release by THP1 cells (Vey *et al. Eur. J. Imm. 26,* 2404-2409, 1996). Serine proteinases have been implicated in the activation of macrophages (Nakabo *et al. J. Leukocyte Biol. 60,* 328-336, 1996), in nuclear laminin degradation in apoptosis (McConkey *et al. J.Biol. Chem., 271,* 22398-22406, 1996), in prostaglandin-E2 induced release of soluble TNF receptor shedding (Choi *et al. Cellular Immunology 170,* 178-184, 1996), in PAF synthesis (Bussolino *et al. Eur. J. Immunol. 24,* 3131-3139, 1994), and in the proteolysis of IkB, a regulatory molecule important in signal transduction and apoptosis. Release of serine proteinases known as granzymes is central to CTL killing and many of the substrates cleaved by granzymes are also cleaved by cellular proteinases (for example, IL-1β is a substrate for Granzyme B as well as the cysteine proteinase, interleukin 1β-converting enzyme (ICE)). Granzyme A, a serine proteinase with Arg-amidolytic activity, has been reported to induce the production of IL-6 and IL-8 in lung fibroblasts (Sower *et al. Cellular Immunology 171,* 159-163, 1996) and cleaves IL-1β to a 17kD mature form that is biologically active.

Kinases are a large group of molecules, many of which regulate the response of cells to external stimuli. These molecules regulate proliferation and differentiation in eukaryotic cells frequently *via* signal transduction pathways.

The identification of new serine proteinases and kinases permits the development of a range of derivatives, agonists and antagonists at the nucleic acid and protein levels which in turn have applications in the treatment and diagnosis of a range of conditions such as cancer, inflammation, neurological disorders amongst many other conditions including conditions which initiate or promote apoptosis such as viral infection, old age and drug abuse. One particularly useful serine

proteinase HELA2 (testisin) identified in accordance with the present invention is involved in spermatogenesis, testicular cancer and as a marker for cancer.

SUMMARY OF THE INVENTION

Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined at the end of the subject specification.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

One aspect of the present invention provides a novel molecule in isolated form involved in or associated with regulation of cell activity and/or viability.

Another aspect of the present invention contemplates an isolated proteinaceous molecule involved in or associated with regulation of cell activity and/or viability comprising a sequence of amino acids encoded by a nucleotide sequence, at least a portion of which, is capable of being amplified by polymerase chain reaction (PCR) using the following primers:

5′ ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3′ [SEQ ID NO:1]; and

5′ ACAGAATTCAXIGGICCICCIC/GT/AXTCICC3′ [SEQ ID NO:2];

or a complementary form of said primers.

The proteinaceous molecule of the present invention may be a serine proteinase or a kinase.

Yet another aspect of the present invention is directed to an isolated serine proteinase comprising the amino acid sequence substantially set forth in SEQ ID NO:4 or an amino acid sequence

having at least about 50% similarity to all or part thereof. This serine proteinase is referred to herein as a short isoform (S) of "HELA2" or "testisin".

Still another aspect of the present invention relates to an isolated serine proteinase comprising the amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least about 50% similarity to all or part thereof. This serine proteinase is referred to herein as a long isoform (L) of HELA2 (testisin).

Still yet another aspect of the present invention provides an isolated serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity to all or part thereof. This serine proteinase is referred to herein as "ATC2".

Even yet another aspect of the present invention is directed to a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions at 42°C.

Another aspect of the present invention relates to a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions at 42°C.

Still another aspect of the present invention provides a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:7 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:7 under low stringency conditions at 42°C.

Another embodiment of the present invention is directed to a kinase in isolated form comprising an amino acid sequence substantially as set forth in SEQ ID NO:10 or having 50% amino acid similarity to all or part thereof. This kinase is referred to herein as "BCON3".

In a related embodiment, the kinase comprises an amino acid sequence encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity to all or part of the nucleotide sequence set forth in SEQ ID NO:9 or a nucleotide sequence capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:9 under low stringency conditions at 42°C.

The present invention further provides an isolated nucleic acid molecule encoding a polypeptide wherein at least a portion of said nucleic acid molecule is capable of being amplified by polymerase chain reaction (PCR) using the following primers:

5′ ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3′ [SEQ ID NO:1]; and

5′ACAGAATTCAXIGGICCICCIC/GT/AXTCICC3′ [SEQ ID NO:2];

or a complementary form of said primers.

The present invention also provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:3 under low stringency conditions at 42°C.

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:5 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:5 under low stringency conditions at 42°C.

Still another aspect of the present invention is directed to an isolated nucleic acid molecule

comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:7 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:7 under low stringency conditions at 42°C.

Even still another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:9 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:9 under low stringency conditions at 42°C.

Another aspect of the present invention provides an isolated serine proteinase encoded by a gene proximal to a cluster of genes on a mammalian chromosome.

More particularly, this aspect of the present invention is directed to a serine proteinase encoded by a gene proximal to a cluster of genes or human chromosome 16p13.3 or its equivalent in a non-human species.

Still more particularly, the serine proteinase is encoded by a gene comprising a nucleotide sequence substantially as set forth in SEQ ID NO:3 or 5 or 28 or 29 or 30 or a nucleotide sequence having at least 50% similarity to any one thereof or a nucleotide sequence capable of hybridizing to any one of SEQ ID NO:3 or 5 or 28 or 29 or 30 under low stringency conditions at 42°C or a nucleotide sequence encoding a serine proteinase having an amino acid sequence substantially as set forth in SEQ ID NO:4 or 6 or an amino acid sequence having at least about 50% similarity to SEQ ID NO:4 or 6.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a representation showing (A) schematic and (B) hydrophobicity plot of the HELA2 amino acid sequence.

Figure 2 is a diagrammatic representation showing: (A) the amino acid sequence of HELA2 (testisin). The putative signal sequence, light chain, heavy chain and transmembrane domains are as indicated, the catalytic amino acids, His, Asp and Ser are as designated; insertion of Tyr-Ser (YS) 4 amino acids after the catalytic His is found in the long isoform of testisin and is due to alternative mRNA splicing; (B) *in vitro* transcription/translation of HELA2 (testisin) showing the protein product.

Figure 3 is a diagrammatic representation of plasmid constructs pBluescriptHELA2(S) and pBluescriptHELA2(L) containing full length cDNAs for testisin (short isoform (S)) and testisin (long isoform (L)), respectively.

Figure 4 is a diagrammatic representation of plasmid constructs pQET(20-295)N and pQET(20-295)C, wherein the hydrophobic residues of testisin were removed and the remaining sequences cloned into pQE prokaryotic expression plasmids; plasmids pGEX-1 (90-279) comprising a carboxy terminal part of testisin fused to glutathione-S-transferase.

Figure 5 is a photographic representation of: (A) silver stained gel showing purification of recombinant HELA2 (testisin) from *E. coli*. The purified HELA2 (testisin) is indicated by the arrow in the eluate fractions. Some HELA2 (testisin) is also found in the wash fractions as the affinity matrix was not used in excess. His-N21 is one clone containing the amino-terminal His tag, and clones His-C21, His-C22 and His-C23 are three different clones with the carboxy-terminal His tag. (B) Western blot of native and denatured recombinant HELA2 (testisin) probed with Clontech anti-His tag-antibody. The 32kD band shown by the arrow is HELA2 (testisin). HELA2 (testisin) is not detected in the denatured samples as it appears that denaturation with urea destroys the His epitope recognised by the monoclonal antibody.

Figure 6 is a representation of the amino acid sequence of HELA2 (testisin) showing the regions of the molecule selected for generation of peptide antigens.

Figure 7 is a photographic representation of a Western blot of GST-HELA2 (testisin) fusion protein purified by affinity chromatography.

Figure 8 is a diagrammatic representation of eukaryotic expression constructs, pcDNA3-Test(S-C), pcDNA3-Test(L-C) and pcDNA3-Test(1-297)L-C.

Figure 9 is a diagrammatic representation showing a histogram of the signal intensity from a Clontech Master RNA blot of the tissue distribution of HELA2 (testisin) in RNA from 50 different normal tissues. (A) Probed with HELA2 (testisin) specific probe; (B) Probed with BCON3 specific prove which is ubiquitously expressed. The 8 tissues on the right hand side of the diagram are the control (negative) samples.

Figure 10 is a photographic representation of a multiple normal tissue Northern blot (Clonetech) probed with: (A) HELA2 (testisin) specific probe and (b) BCON3 specific probe.

Figure 11 is a photographic representation of agarose gel of PCR products generated by amplification of HELA2 (testisin) cDNA in prevasectomised and post-vasectomised ejaculate specimens. The HELA2 (testisin) PCR product is 464bp and the β2-macroglobulin product is 250 bp.

Figure 12 is a photographic representation of *in situ* hybridization of rat testis showing the localisation of HELA2 (testisin) mRNA to the germ cells of the testis.

Figure 13 is a representation showing: (A) spread of normal metaphase chromosomes showing bright dots where HELA2 (testisin) is expressed at 16p13.3; (B) Diagrammatic representation of chromosome 16p13.3 showing location of HELA (testisin) and relationship to other disease causing genes.

Figure 14 is: (A) a photographic representation of northern blot analysis of HELA2 (testisin) mRNA showing signals in normal testis of 4 patients and absence of signal in the tumours of these patients; (B) a photographic representation of the localisation of HELA2 (testisin) protein in a human germ cell tumour section assessed by immunohistochemical staining using anti-HELA2 (testisin) peptide antibodies. Staining is only detected in the normal (N) tissue and not present in the tumour (T) tissue.

Figure 15 is a diagrammatic representation of the genomic map of HELA2 (testisin) showing experimentally determined intron/exon boundaries and relative sizes of the introns (marked with a letter) and exons (marked with a roman numeral).

Figure 16 is a representation of HELA2 (testisin). Nucleotides in introns are in lowercase and exons in uppercase. The putative transcription start site is marked by +1.

Figure 17 is a representation of the DNA sequence of Intron C and flanking exons showing where alternative mRNA splicing occurs to generate the two isoforms of HELA2 (testisin).

Figure 18 is a representation of: (A) the cDNA sequence of the mouse homologue of HELA2 (testisin). Catalytic residues are indicated by circles and cysteines likely involved in disulfide bonding are indicated by squares; (B) Hydrophobicity plot of HELA2 (testisin) amino acid sequence.

Figure 19 is a diagrammatic representation of chromosome 16p13.3 showing the serine proteinase gene cluster which includes HELA2 (testisin). Lines represent cosmids containing the respective serine proteinase genes.

Figure 20A is a representation of: (A) the cDNA sequence of SP001LA (SEQ ID NO:28). Catalytic residues are indicated by circles and cysteins likely involved in disulfide bonding are indicated by squares; (B) hydrophobicity plots of SP001LA amino acid sequence.

Figure 20B is a representation of: (A) the cDNA sequence of SP002LA (SEQ ID NO:29).

Catalytic residues are indicated by circles and cysteines likely involved in disulfide bonding are indicated by squares. (B) Hydrophobicity plot of SP002LA amino acid sequence.

Figure 20C is a representation of: (A) the cDNA sequence of SP003LA (SEQ ID NO:30). Catalytic residues are indicated by circles and cysteines likely involved in disulfide bonding are indicated by squares. (B) Hydrophobicity plot of SP003LA amino acid sequence.

Figure 21 is a photographic representation of *in vitro* transcription/translation of BCON3 showing the protein products.

A summary of the SEQ ID NOs used throughout the specification is presented in Table 1.

TABLE 1

SEQ ID NO	DESCRIPTION
1	* PCR primer sequence
2	* PCR primer sequence
3	Nucleotide sequence of short form of HELA2
4	Amino acid sequence of short form of HELA2
5	Nucleotide sequence of long form of HELA2
6	Amino acid sequence of long form of HELA2
7	Nucleotide acid sequence of ATC2
8	Amino acid sequence of ATC2
9	Nucleotide acid sequence of BCOM3
10	Amino acid sequence of BCOM3
11	Primers used to generate amino terminal tagged protein
12	Primers used to generate amino terminal tagged protein
13	Primers used to generated carboxy-linked terminal protein
14	Primers used to generated carboxy-linked terminal protein
15	Peptide antigen T20-33
16	Peptide antigen T46-63
17	Peptide antigen T175-190
18	Forward primer
19	Reverse primer
20	Forward primer
21	Reverse primer

TABLE 1 (Continued)

22	Forward primer
23	Reverse primer
24	Serine proteinase activation motif
25 & 26	Mouse HELA2 cDNA sequence
27	Human genomic DNA sequence
28	Clustered serine proteinase gene SP001LA
29	Clustered serine proteinase gene SP002LA
30	Clustered serine proteinase gene SP003LA

* Abbreviations: X= A or G

Y= C or T

I= Inosine.

A list of single and three letter abbreviations for amino acid residues is presented in Table 2.

TABLE 2

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention is predicated in part on a genetic engineering approach to identify nucleotide sequences encoding serine proteinases or kinases. The genetic engineering approach is based on the use of degenerate primers corresponding to conserved regions of serine proteinases (amino acids flanking His- and Ser- residues) to amplify gene fragments spanning these regions for cDNA, using low stringency reverse transcriptase-polymerase chain reaction (RT-PCR).

This technique has been successfully used, in accordance with the present invention, to identify serine proteinases and kinases useful in modulating cell activity and viability including modulating spermatogenesis, acting as tumour suppressors and acting as a marker for non-testicular cancers.

Accordingly, one aspect of the present invention provides a novel molecule in isolated form involved in or associated with regulation of cell activity and/or viability.

More particularly, the present invention contemplates a novel serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence, at least a portion of which, is capable of being amplified by polymerase chain reaction (PCR) using the following primers:

5′ ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3′ [SEQ ID NO:1]; and

5′ACAGAATTCAXIGGICCICCIC/GT/AXTCICC3′ [SEQ ID NO:2];

or a complementary form of said primers.

Preferably, X is A or G, Y is C or T and I is inosine.

In a particularly preferred embodiment, the isolated serine proteinase comprises the amino acid sequence substantially set forth in SEQ ID NO:4 or an amino acid sequence having at least about

50% similarity to all or part thereof. This serine proteinase is referred to herein as a short isoform of "HELA2" or "HELA2 (testisin)". The terms "HELA2" and "testisin" are used interchangedly throughout the subject specification to refer to the same molecule.

In another preferred embodiment, the amino acid sequence of the serine proteinase is substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least about 50% similarity to all or part thereof. This serine proteinase is the long isoform of HELA2 or HELA2 (testisin).

Yet another preferred embodiment of the present invention provides an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity to all or part thereof. This serine proteinase is referred to herein as "ATC2".

Another aspect of the present invention relates to a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions at 42°C.

Still another aspect of the present invention is directed to a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions at 42°C.

In another aspect of the present invention, there is provided a serine proteinase in isolated form comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:7 or a nucleotide sequence having at least 50% similarity to all or part thereof or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:7 under low stringency conditions at 42°C.

Another embodiment of the present invention is directed to a kinase in isolated form comprising an amino acid sequence substantially as set forth in SEQ ID NO:10 or having 50% amino acid similarity to all or part thereof. This kinase is referred to herein as "BCON3".

In a related embodiment, the kinase comprises an amino acid sequence encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity to all or part of the nucleotide sequence set forth in SEQ ID NO:9 or a nucleotide sequence capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:9 under low stringency conditions at 42°C.

The present invention further provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a novel molecule involved in or associated with regulation of cell activity and/or viability. Preferably, the nucleic acid molecule is capable of being amplified by PCR using the primers set forth in SEQ ID NO:1 and/or SEQ ID NO:2.

More particularly, the present invention further provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:3 under low stringency conditions at 42°C.

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:5 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:5 under low stringency conditions at 42°C.

Another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:7 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:7 under low stringency conditions at 42°C

Still another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:9 or having 50% similarity to all or part thereof or a nucleic acid molecule capable of hybridising to SEQ ID NO:9 under low stringency conditions at 42°C.

Reference herein to a low stringency includes low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

Reference herein to similarity to "part" of a sequence means similarity to at least about 4 contiguous amino acids or at least about 12 contiguous nucleotide bases and more preferably at least about 7 contiguous amino acids or at least about 21 contiguous nucleotide bases.

The term "similarity" includes exact identity between sequences or, where the sequence differs, different amino acids may be related to each other at the structural, functional, biochemical and/or conformational levels.

The term "isolated" includes biological purification and biological separation and encompasses molecules having undergone at least one purification, concentration or separation step relative to its natural environment. For example, a preparation may comprise at least about 10%, preferably at least about 20%, more preferably at least about 30%, still more preferably at least about 50% or greater of the molecule relative to at least one other component in a composition as determined by activity, mass, amino acid content, nucleotide content or other convenient means.

Hereinafter, the molecules of the present invention are referred to as a "proteinase/kinase". The term "proteinase/kinase" includes the serine proteinases HELA2 (testisin) and ATC2 and the kinase BCON3. The proteinase/kinase of the present invention may be in isolated, naturally occurring form or recombinant or synthetic form or chemical analogues thereof.

The proteinase/kinase of the present invention is preferably of human origin but from non-human origins are also encompassed by the present invention. Non-human animals contemplated by the present invention include primates, livestock animals (e.g. sheep, cows, pigs, goats, horses, donkeys), laboratory test animals (e.g. mice, rats, guinea pigs, hamsters, rabbits), domestic companion animals (e.g. dogs, cats), birds (e.g. chickens, geese, ducks and other poultry birds, game birds, emus, ostriches) and captive wild or tamed animals (e.g. foxes, kangaroos, dingoes). The present invention also encompasses a proteinase/kinase homologue from *Xenopus* and plants.

The nucleic acid molecules encoding a proteinase/kinase may be genomic DNA, cDNA or RNA such as mRNA.

Yet another aspect of the present invention provides an isolated serine proteinase encoded by a gene proximal to a cluster of genes on a mammalian chromosome. The cluster of genes is preferably on human chromosome 16p13.3 or its equivalent in a non-human species. The cluster is made up of genes all encoding or having the potential to encode a serine proteinase or homologue, derivative or functional or evolutionary equivalent thereof. Preferably, the gene cluster comprises two or more of genes comprising a nucleotide sequence selected from SEQ ID NO:3 and 5 (HELA2, short and long forms, respectively) and SEQ ID NO:28 (SP001LA), SEQ ID NO:29 (SP002LA), SEQ ID NO:30 (SP003LA) and SP004LA (see Figure 19) or a nucleotide sequence having at least 50% similarity to any one of those sequences or capable of hybridizing to any one of those sequences under low stringency conditions at 42°C.

The term "proximal" is used in its broadest sense to mean a gene cluster and includes a gene within proximity to another gene.

Another aspect of the present invention contemplates a method for cloning a nucleotide sequence encoding a novel serine proteinase, said method comprising screening a nucleic acid library with said one or more or oligonucleotides defined by SEQ ID NO:1 and/or SEQ ID NO:2 and obtaining a clone therefrom which encodes said novel serine proteinase or part thereof.

Preferably, the nucleic acid library is genomic DNA, cDNA, genomic or mRNA library.

Preferably, the nucleic acid library is a cDNA expression library.

Preferably, the nucleic acid library is of human origin such as from brain, liver, kidney, neo-natal tissue, embryonic tissue, tumour or cancer tissue.

With respect to HELA2 (HELA2 (testisin)), significant expression is generally only found in normal testis. Accordingly, the present invention extends to nucleic acid molecules capable of tissue-specific or substantially tissue-specific expression.

Still another embodiment contemplates the promoter or a functional part thereof of the genomic gene encoding the subject proteinase/kinase of the present invention. The promoter may readily be obtained by, for example, "chromosome walking". A particularly useful promoter is from HELA2 (testisin) which can be regarded as a testis specific promoter. This promoter can be used, for example, to direct testis specific expression of genetic sequences operably linked to the promoter and may be used *inter alia* gene therapy or modulation of fertility.

The present invention further contemplates a range of derivatives of the subject proteinase/kinase. Derivatives include fragments, parts, portions, mutants, homologues and analogues of the subject polypeptides and corresponding genetic sequences. Derivatives also include single or multiple amino acid substitutions, deletions and/or additions to the subject molecules or single or multiple nucleotide substitutions, deletions and/or additions to the genetic sequence encoding the molecules. "Additions" to amino acid sequences or nucleotide sequences include fusions with other peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to the serine proteinase and kinase includes reference to all derivatives thereof

including functional derivatives or immunologically interactive derivatives.

Analogues of the subject serine proteinase and kinase contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides.

Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 3.

TABLE 3

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva

D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-çylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp

D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr

L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

These types of modifications may be important to stabilise the proteinase/kinase if administered to an individual or for use as a diagnostic reagent.

The present invention further contemplates chemical analogues of the proteinase/kinase capable of acting as antagonists or agonists of the native molecules or which can act as functional analogues of the native molecules. For example, an antagonist may be a proteinase inhibitor. Chemical analogues may not necessarily be derived from the subject enzymes but may share certain conformational similarities. Alternatively, chemical analogues may be specifically designed to mimic certain physiochemical properties of the serine proteinases or kinases. Chemical analogues may be chemically synthesised or may be detected following, for example, natural product screening.

The identification of the novel molecules of the present invention permits the generation of a

range of therapeutic molecules capable of modulating expression of their native counterparts or modulating their activity. Modulators contemplated by the present invention includes agonists and antagonists of proteinase/kinase expression. Antagonists of proteinase/kinase expression include antisense molecules, ribozymes and co-suppression molecules. Agonists include molecules which increase promoter ability or interfere with negative regulatory mechanisms. Agonists of proteinase/kinase include molecules which overcome any negative regulatory mechanism. Antagonists of the proteinase/kinase include antibodies and inhibitor peptide fragments.

Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

Another embodiment of the present invention contemplates a method for modulating expression of proteinase/kinase in a human, said method comprising contacting the proteinase/kinase gene encoding proteinase/kinase with an effective amount of a modulator of proteinase/kinase expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of proteinase/kinase. For example, a nucleic acid molecule encoding proteinase/kinase or a derivative thereof may be introduced into a cell conversely, proteinase/kinase antisense sequences such as oligonucleotides may be introduced.

Another aspect of the present invention contemplates a method of modulating activity of proteinase/kinase in a human, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease proteinase/kinase activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative of proteinase/kinase or its receptor or a chemical analogue or truncation mutant of proteinase/kinase or its receptor.

One particularly useful serine proteinase, HELA2 (testisin), is implicated in spermatogenesis and in testicular tumour development. It is proposed, in accordance with the present invention,

that HELA2 (testisin) is involved in fertility and infertility.

Northern blot analysis of Poly A+ RNA from normal tissue specimens showed a unique tissue distribution for HELA2 (testisin) with significant expression only in the testis. No signals are detected in any other tissue, with the exception of a minor signal in salivary gland. By RT-PCR, HELA2 (testisin) is detected in the ejaculate of normal males but not in the ejaculate of vasectomised males indicating that it is of germ cell origin. Hybridization data *in situ* indicated that HELA2 (testisin) is produced by immature germ cells in the testis, located near the basal epithelium and, hence, is an important factor for normal sperm maturation; defective expression or mutations would contribute to primary male infertility. Further, it is from the precursors of spermatocytes that 95% of testicular germ cell tumours, such as seminomas, embryonal carcinomas and teratocarcinomas arise. In the normal testis, germ cells undergo meiosis to become spermatocytes, but in individuals at risk, the germ cells continue to proliferate giving rise to germ cell tumours. Although not wishing to limit the present invention to any one theory or mode of action, it is proposed, in accordance with present invention, that HELA2 (testisin) functions at this critical juncture - cell growth versus maturation.

Familial forms of testicular cancer are rare, but linkage analysis of a large family with familial seminoma has demonstrated linkage to chromosome 16p, within a region adjacent to the HPKD1 (human polycystic kidney disease) gene at 16p13.3. The HELA2 (testisin) gene localises to chromosome 16p13.3 which is near the telomere of chromosome 16 and is associated with high genetic instability. The HELA2 (testisin) gene is sandwiched between four genes which underlie other human genetic disorders; HPKD1 and tuberous sclerosis (TSC2) on the one side, and familial mediterranean fever (MEF) and Rubenstein-Taybi syndrome (RSTS) on the other side. The question of whether HELA2 (testisin) may be a tumour suppressor for seminoma was determined by comparing HELA2 (testisin) mRNA expression in normal testes with corresponding germ cell tumours from patients with seminoma. HELA2 (testisin) was not detectable in the tumours of these patients, but was present in the corresponding normal testis specimens, indicative of a tumour suppressor role of HELA2 (testisin) in testicular germ cell cancers.

Although restricted in normal tissues to the testes, HELA2 (testisin) is expressed in tumours of the colon, pancreas, prostate and ovary. This indicates that HELA2 (testisin) contributed to tumourigenesis and, therefore, has an application as a marker and also as a therapeutic anti-tumour target in these types of cancers.

These data point to a potentially very significant role for HELA2 (testisin) in testicular germ cell maturation (spermatogenesis) as well as in the genesis of testicular germ cell tumours. In accordance with the present invention, it is proposed that expression of HELA2 (testisin) by immature germ cells may be essential for sperm cell development, such that loss of HELA2 (testisin) expression leads to continued and uncontrolled proliferation of immature germ cells leading to subsequent tumourigenesis. Germ cells wherein HELA2 (testisin) is mutated or absent may thus be prone to malignant transformation because of an inability to progress along the differentiation pathway.

HELA2 (testisin) is well-positioned to anchor on the surface of the germ cell where it would participate in a range of proteolytic activities, including cell migration, differentiation and/or activation of growth factors, receptors, or cytokines as well as initiate additional proteolytic cascades. Although not intending to limit the present invention to any one theory or mode of action, it is proposed, in accordance with the present invention, that the proteolytic target of HELA2 (testisin) is a cytokine, receptor or growth factor essential for either germ cell proliferation or differentiation - ie. HELA2 (testisin) may either inactivate a factor important for proliferation, or activate a factor which promotes differentiation. Thus, HELA2 (testisin) may be critical in the regulation of specific cytokines, cytokine receptors or growth factors by means of post-translational proteolytic processing. That HELA2 (testisin) is not present in other normal tissues of the male urogenital tract, such as the prostate and kidney, also argues for such a role specific to the testis.

Diagnostic and therapeutic applications for HELA2 (testisin) have the potential to be wide-ranging both in the cancer and fertility/infertility markets. In tumours, other than the testis, it is desirable to block or inhibit HELA2 (testisin) activity. As HELA2 (testisin) is a member of the serine proteinase family, for which prototype crystal structures are known and the catalytic

mechanism reasonably well characterised, the design of drugs that target HELA2 (testisin) proteolytic activity as an anti-tumour therapy should be relatively straightforward. As HELA2 (testisin) is predicted to be anchored on the cell surface, there would not be difficulties associated with delivery of drugs to intracellular compartments. Further, it is very possible that some tumour-associated HELA2 (testisin) may be proteolytically cleaved from the surface of tumour cells, and the extracellular domain detectable in patient serum as a potential tumour associated marker.

Testicular cancer is the commonest malignancy in men aged 20-44 years. Early diagnosis correlates which an improved chance of cure and in a reduction in the severity of treatment. If the cancer is not treated early, it becomes very aggressive. The incidence of testicular cancer is significant (9/100,000) and has been rising over the last 10 years. In testicular germ cell tumours, such as seminoma, delivery of recombinant HELA2 (testisin) using gene therapy techniques could lead to arrest of tumour growth and potentially allow commencement of normal sperm cell maturation and differentiation, thereby reducing the need for surgical removal of the testis (orchidectomy). This may be particularly effective for patients who have already had one testicle removed because of testicular cancer. The risk of contralateral testicular cancer is increased in these patients and tumour development could be arrested through early treatment with HELA2 (testisin) to arrest growth and assist maturation of germ cells. The finding of mutant forms of HELA2 (testisin) may also lead to new markers for seminoma. Unlike other testicular non-seminoma cancers where α-fetoprotein and β-HCG are frequently elevated and can be used as tumour markers, the lack of an adequate marker for seminoma creates difficulties with staging and patient follow-up.

A demonstrated role for HELA2 (testisin) in sperm maturation and development would likely lead to improved diagnosis and new directed therapeutics for male primary infertility. Primary male infertility is responsible for conception problems in 5-10% of couples and the world market for a therapeutic in this area would be very substantial. Delivery of recombinant HELA2 (testisin) could assist sperm maturation and potentially trigger normal sperm development in some of these cases. The identification of mutant forms of HELA2 (testisin) could aid in diagnosis of infertility. If HELA2 (testisin) does not prove to be a tumour

suppressor, but is important for sperm maturation, it could provide a new target for the development of a male contraceptive. If hormonal regulation of HELA2 (testisin) can be demonstrated, HELA2 (testisin) may prove effective for the treatment of conditions arising from dysfunctional hormal responses, such as cryptorchidism, which is associated with both infertility and seminoma development.

Accordingly, the present invention contemplates a pharmaceutical composition comprising proteinase/kinase or a derivative thereof or a modulator of proteinase/kinase expression or proteinase/kinase activity and one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to as the "active ingredients" and include, for example, HELA2 (testisin).

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion

medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter. A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release

preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Parental compositions are generally suitable for administration by the intravenous, subcutaneous or intramuscular routes amongst other routes of administration. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail. Other forms of administration include but are not limited to intranasal, buccal, rectal, suppository, inhalation, intracerebral and intraperitoneal.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The effective amounts include amounts calculated or predicted to have the desired effect and range from at least about 0.01 ng/kg body weight to about 10,000 mg/kg body weight. Alternative amounts include 0.1 ng/kg body weight to about 1000 ng/kg body weight.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating proteinase/kinase expression or proteinase/kinase activity. The vector may, for example, be a viral vector. This form of therapy is proposed to be particularly useful for gene replacement or enhancement therapy for HELA2 (testisin) especially for the modulation of fertility and/or treatment of testicular cancer.

Still another aspect of the present invention is directed to antibodies to proteinase/kinase and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to proteinase/kinase or may be specifically raised to proteinase/kinase or derivatives thereof. In the case of the latter, proteinase/kinase or its derivatives may first need to be associated with a carrier molecule. The antibodies and/or recombinant proteinase/kinase or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents. For example, monitoring non-testicular cancer by measuring HELA2 (testisin) or screening for the presence of testicular cancer by an absence of HELA2 (testisin).

Proteinase/kinase and its derivatives may also be used to screen for naturally occurring antibodies to proteinase/kinase. These may occur, for example in some autoimmune diseases. Alternatively, specific antibodies can be used to screen for proteinase/kinase. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of proteinase/kinase levels may be important for diagnosis of certain cancers or a predisposition to cancers or for monitoring certain therapeutic protocols.

Antibodies the proteinase/kinase of the present invention may be monoclonal or polyclonal. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A

"synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a diagnostic tool for assessing apoptosis or monitoring the program of a therapeutic regimen.

For example, specific antibodies can be used to screen for proteinase/kinase proteins. The latter would be important, for example, as a means for screening for levels of proteinase/kinase in a cell extract or other biological fluid or purifying proteinase/kinase made by recombinant means from culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of proteinase/kinase.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of proteinase/kinase, or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques

which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates a method for detecting proteinase/kinase in a biological sample from a subject said method comprising contacting said biological sample with an antibody specific for proteinase/kinase or its derivatives or homologues for a time and under conditions sufficient for an antibody-proteinase/kinase complex to form, and then detecting said complex.

The presence of proteinase/kinase may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These, of course, includes both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain proteinase/kinase including cell extract,

tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

In the typical forward sandwich assay, a first antibody having specificity for the proteinase/kinase or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from about room temperature to about 37°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most

commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionucleotide containing molecules (i.e. radioisotopes) and chemiluminescent molecules. In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescene and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

The present invention also contemplates genetic assays such as involving PCR analysis to detect proteinase/kinase gene or its derivatives. Alternative methods or methods used in conjunction include direct nucleotide sequencing or mutation scanning such as single stranded conformation polymorphoms analysis (SSCP), specific oligonucleotide hybridisation, and methods such as direct protein truncation tests.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli, Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and a mammalian and more particularly a human proteinase/kinase gene portion, which proteinase/kinase gene portion is capable of encoding an proteinase/kinase polypeptide or a functional or immunologically interactive derivative thereof.

Preferably, the proteinase/kinase gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said proteinase/kinase gene portion in an appropriate cell.

In addition, the proteinase/kinase gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

The present invention also extends to any or all derivatives of proteinase/kinase including mutants, part, fragments, portions, homologues and analogues or their encoding genetic sequence including single or multiple nucleotide or amino acid substitutions, additions and/or deletions to the naturally occurring nucleotide or amino acid sequence. The present invention further encompasses hybrids between the proteinase/kinases such as to broaden the spectrum of activity and to ligands and substrates of the proteinase/kinase.

The proteinase/kinase and its genetic sequence of the present invention will be useful in the generation of a range of therapeutic and diagnostic reagents.

Soluble proteinase/kinase polypeptides or other derivatives, agonists or antagonists are also contemplated to be useful in the treatment of disease, injury or abnormality in the nervous system, e.g. in relation to central or peripheral nervous system to treat Cerebral Palsy, trauma induced paralysis, vascular ischaemia associated with stroke, neuronal tumours, motoneurone disease, Parkinson's disease, Huntington's disease, Alzheimer's disease, Multiple Sclerosis, peripheral neuropathies associated with diabetes, heavy metal or alcohol toxicity, renal failure and infectious diseases such as herpes, rubella, measles, chicken pox, HIV or HTLV-1. Other conditions for which the proteinase/kinase are useful include cancer, metastasis and autoimmune disease amongst many others. Particular applications for HELA2 (testisin) include as a marker for non-testicular cancers, in the treatment of testicular cancer and in the treatment of infertility or in inducing infertility such for contraception.

A further aspect of the present invention contemplates the use of proteinase/kinase or its functional derivatives in the manufacture of a medicament for the treatment of proteinase/kinase mediated conditions defective or deficient.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1
CLONING PROCEDURES

In order to identify serine proteinases that may be involved in regulatory cellular functions, a genetic screening approach was applied using degenerate primers corresponding to conserved regions of serine proteinases (amino acids flanking His- and Ser- residues) to amplify gene fragments spanning these regions from cDNA, using a low stringency RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) approach.

By this technique, the aim was to isolate low abundance genes as well as those present in moderate to high abundance. The cDNA used for these experiments was isolated from a HeLa cell cytotoxicity model wherein PAI-2 expression inhibits TNF(-induced apoptosis (Dickinson *et al J. Biol. Chem. 270:* 27894-27904, 1995). These PAI-2 expressing cells provide a unique and viable system for investigating TNF(signalling pathways as they are protected from the cytotoxic effects of TNF).

cDNA was generated from RNA isolated from HeLa cells and PAI-2 expressing HeLa cells, both untreated and following treatment with TNF and cycloheximide. Amplification of each cDNA population using PCR and the following serine proteinase degenerate primers,

His Primer: 5'ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3' [SEQ ID NO:1],
Ser Primer: 5'ACAGAATTCAXIGGICCICCIC/GT/AXTCICC3' [SEQ ID NO:2]

(where X= A or G; Y= C or T; I= Inosine)
produced DNA fragments in the range of 480bp, the approximate predicted size of the serine proteinase intergenic region. These amplified DNA fragments were cloned into E. coli generating a library containing approximately 150 independent clones. The inventors analysed 36 of these clones and found that 9 encoded previously identified serine proteinases or tissue-type or urokinase-type plasminogen activators, thereby demonstrating the efficacy of this approach. Of the other 36, two were found to encode novel open reading frames with high homology to serine proteinases and are referred to herein as "HELA2" (or "testisin") and

"ATC2". One additional clone designated herein, "BCON3", showed homology to a kinase. Extension of the DNA fragments by RACE in both 5' and 3' directions using internally derived primers has verified the homology of HELA2 and ATC2 to the serine proteinase family. Each of the three DNA sequences are unique in that they are markedly different from any known DNA or protein sequence in the Genbank and NBRF databases.

EXAMPLE 2
HELA2 SERINE PROTEINASE (TESTISIN)

The HELA2 mRNA transcript is approximately 1.5kb as determined from Northern blot analysis. Nucleic acid sequence was obtained for about 1.1kb of HELA2 which spans the entire coding region, the 3' noncoding region and part of the 5' noncoding region. The coding region starts with an ATG codon which is present in a motif analogous to the Kozak eukaryotic translation initiation consensus sequence. Alignment of the deduced amino acid sequence of HELA2 with homologous serine proteinases shows that the cDNA encodes a 314 amino acid (aa) polypeptide with a calculated molecular weight of 34.8kD (called Testisin), which is synthesized as a zymogen containing pre-, pro- and catalytic regions (Figure 1). The pro- region (or light chain) and the catalytic region (heavy chain) are delineated by a classic serine proteinase activation motif Arg-Ile-Val-Gly-Gly [SEQ ID NO:24] with cleavage likely occurring between Arg and Ile. The catalytic region includes the catalytic triad of His, Asp and Ser in positions and motifs which are highly conserved among the serine proteinases. Ten Cys residues occur in conserved positions: by analogy to other serine proteinases, eight of these function to form disulfide bridges within the catalytic region and the remaining two link the pro- and catalytic regions.

Structural features conserved in the binding pockets of serine proteinases are present in HELA2 (testisin). An Asp residue at the bottom of the serine proteinase binding pocket six residues before the active site Ser in HELA2 (testisin) indicates that HELA2 (testisin) has trypsin-like specificity, with proteolytic cleavage after Arg or Lys in target substrates. HELA2 (testisin) also contains a conserved Ser-Trp-Gly motif at the top of the binding pocket which is likely involved in hydrogen bonding with target substrates in other serine proteinases.

A hydrophobicity plot of the HELA2 (testisin) amino acid sequence (Figure 1) identifies two hydrophobic regions, one located at the amino terminus and the other at the carboxy terminus. The 20 aa amino terminal hydrophobic region is likely to be a signal peptide, which would direct newly synthesized HELA2 to enter the endoplasmic reticulum. The 16 aa hydrophobic carboxy terminus of HELA2 (testisin) shows high homology to the transmembrane domain of prostasin (Figure 2), suggesting that HELA2 (testisin) is likely to be a membrane-anchored serine proteinase. Thus HELA2 (testisin) may anchor on the germ cell surface where it could participate in a range of proteolytic activities, including participation in cell migration, differentiation and/or activation of growth factors and proteolytic cascades. In prostasin, this protruding carboxy terminus may be cleaved, thus releasing the serine proteinase from the membrane. A similar cleavage event may also occur with Testasin.

Two isoforms of HELA2 were identified in a HeLa cell cDNA library (Stratagene UniZap HeLa Library) which differ by an insertion of 6 nucleotides which generates a Sfi1 restriction enzyme site. At the protein level, there is a corresponding insertion of 2 aa's (Tyr-Ser) within the catalytic binding pocket (Figure 2A). The two isoforms of HELA2 cDNA are referred to as the short (S) and long (L) isoforms, respectively. The nucleotide and corresponding amino acid sequence for the short isoform of HELA2 is shown in SEQ ID NOs. 3 and 4, respectively. The long isoform is shown in SEQ ID NO:5 and 6, respectively.

EXAMPLE 3

GENERATION OF FULL LENGTH cDNA ENCODING HELA2 (TESTISIN)

Partial cDNA fragments of the short and long isoforms of HELA2 were obtained using a combination of library screening techniques. Plasmids containing the full length cDNA of the two isoforms were then generated in pBluescriptSK(-) by ligating restriction enzyme-digested fragments of the partial cDNAs. A plasmid map of the two generated constructs, pBluescriptHELA2(S) and pBluescriptHELA2(L), and a restriction enzyme map of the long isoform cDNA are diagrammed in Figure 3.

In vitro transcription/translation using HELA2 cDNA shows a major specific product of

approximately 35kD (Fig. 2B), which is the same as size predicted from the open reading frame, demonstrating that HELA2 cDNA encodes a protein. The translation/transcription coupled rabbit reticulocyte lysate system (Promega) was used as per the manufacturer's instructions for 35S-methionine labelling. Clones of HELA2 in pBluescript a PAI-2 positive control were used with T3-RNA polymerase (sense direction).

EXAMPLE 4
EXPRESSION OF RECOMBINANT HELA2 (TESTISIN) IN E.COLI

(A) Generation of expression constructs

(i) His(6)-tagged recombinant HELA2 (testisin)

To reduce potential toxic effects on host cells, and therefore optimise expression, a strategy was employed to eliminate the hydrophobic residues of the secretory and membrane anchoring domains of HELA2 (testisin) (Testisin (20-295)). Testisin (20-295) fragments which were His6 tagged at either the amino or carboxy terminal were obtained by PCR and expression constructs were generated by inserting these into pQE vectors (Qiagen).

The primers used to generate the amino-terminal tagged protein were:
forward: 5' GCACAGTCGACCAAGCCGGAGTCGCAGAG 3' [SEQ ID NO:11] and
reverse: 5' GCACAAAGCTTGCCAGGAGGGGTCTGGCTG 3' [SEQ ID NO:12]
The amplification product of 858bp was digested with SalI and HindIII and ligated into pQE-10 to give pQE-10(20-295)N (Figure 4).

The primers used to generate the carboxy-terminal tagged protein were:
forward: 5' GCACAACCATGGCCAAGCCGGAGTCGCAGGAG 3' [SEQ ID NO:13] and
reverse 5' GCACAAGATCTCCAGGAGGGGTCTGGCTG 3' [SEQ ID NO:14].
The amplification product of 859 bp was digested with NcoI and BglII and ligated into PQE-60 to give pQE-60(20-295)C (Figure 4).

(ii) GST-tagged recombinant HELA2 (testisin)

In order to generate a fusion of glutathione-S-transferase (GST) and HELA2 (testisin),

pBluescriptHELA2(S) was digested with Sau3A1, releasing a 570bp DNA fragment encoding the 190 amino acids at the carboxy terminal end of HELA2 (testisin). This DNA fragment was cloned into the BamH1 site of pGEX-1 generating pGEX-1(90-279) (Figure 4) and subjected to DNA sequence analysis to confirm that the fusion was in frame.

(b) Expression of His-tagged HELA2 (testisin) in E. coli

pQE10(20-295)N and pQE60(20-295)C plasmids were electro-transformed into E. coli DH5(cells. Four different clones were selected for further analysis: His-N21 expressing amino terminal His6-tagged Testisin (20-295); and His-C21, His-C22, and His-C23 expressing carboxy terminal His6-tagged Testisin (20-295). To express recombinant HELA2 (testisin) protein, transformed cells were grown to log phase then induced for 4 hours in the presence of 2mM IPTG. Cells were lysed in a denaturing lysis buffer containing 8M urea, 0.1M NaH2PO4 and 0.01M Tris/HCI pH8. Alternatively the cells were lysed in a non-denaturing lysis buffer containing 0.1M NaH2PO4, 0.1M NaCl and 0.01 M Tris/HCI pH8. The His6 tagged protein was recovered by mixing the lysate with a metal affinity resin (Qiagen or Clontech). Purified testisin(L) was eluted with 100 mM EDTA in lysis buffer (pH 6.3). A major band of approximately 32 kDa was obtained in the eluate as shown by the arrows in Figure 5A. Western blot analysis of a purification of the His-C23 clone using an anti-His6 antibody showed that the band at 32 kDa was His6 tagged HELA2 (testisin) (Figure 5B).

EXAMPLE 5
IMMUNOLOGY

(A) Rabbit Polyclonal Antibodies Directed Against HELA2 (testisin) Peptide Antigens

Three peptides were selected from the HELA2 (testisin) amino acid sequence on the basis of predicted antigenicity, hydrophilicity and lack of identity with known proteins (Figure 6).

Peptide antigen T20-33	KPESQEAAPLSGPC [SEQ ID NO:15]
Peptide antigen T46-63	EDAELGRWPWQGSLRLWDC [SEQ ID NO:16]

Peptide antigen T175-190 GYIKEDEALPSPHTLQC [SEQ ID NO:17]

These peptides were synthesized (Auspep) and coupled to keyhole limpet hemocyanin. The coupled peptide (500 Fg) in PBS (0.5 ml) was emulsified in an equal volume of Freund's complete adjuvant before injection into a rabbit. Booster injections of coupled peptide in Freund's incomplete adjuvant were made at intervals of 2 to 3 weeks. Each rabbit was bled (approximately 1 ml) before the initial injection and about 7 days after the second and subsequent boosters and the antibody titre assessed by direct ELISA assay. Immunoreactive antisera against the peptide antigens was demonstrated and when a sufficiently high titre was achieved (after 3 to 5 boosters), between 12 and 25 ml of blood was removed from each animal.

Rabbit antisera was affinity purified against the respective immunising peptides by chromatography using peptide-coupled affinity columns. Immunoreactivity of the affinity purified antibodies against HELA2 (testisin) was demonstrated by Western blot analysis of GST-tagged recombinant HELA2 (testisin). pGEX-1(90-279) plasmid DNA (described in Example 4) was electro-transformed into E. coli DH5(cells and induced for 3 hours in the presence of 0.5mM IPTG. Cells were lysed in 1.5% sarcosyl, 2% Triton X100 and then sonicated. After removal of the insoluble fraction by centrifugation, the cell lysate was mixed with a 50% slurry of Glutathione Sepharose 4B, washed, and the purified GST-Testisin(90-279) was eluted by boiling with SDS-Sample buffer. Figure 7 shows an example of Western blot analysis of the eluate using anti-Peptide T175-190 antibody demonstrating a purified, immunoreactive band representative of GST-linked HELA2(testisin) of approximately 47 kDa.

(B) Rabbit Polyclonal Antibodies Directed Against Purified Bacterially Expressed HELA2 (testisin)

An SDS-PAGE gel slice containing purified His6 tagged HELA2 (testisin) (as described in Example 4, part (b)) is to be combined with adjuvant and rabbits immunized as described above. Rabbit antisera are tested by Western blot analysis for immunoreactivity against purified recombinant HELA2 (testisin) and HELA2 (testisin) in cell extracts, as well as use in immunohistochemical analyses.

EXAMPLE 6

EXPRESSION OF HELA2 (TESTISIN) IN EUKARYOTIC CELLS

(A) Generation of expression constructs

Eukaryotic expression constructs encoding testisin(s) and testisin(L) His6 tagged at the carboxy terminal were generated in the eukaryotic expression vector pcDNA3 (Invitrogen). DNA fragments encoding HELA2 (testisin) were generated by PCR from both pBluescriptHELA2(S) and pBluescriptHELA2(L) using the primers:
forward: 5' GCACAGGTACCGAGGCCATGGGCGCGCGC 3' [SEQ ID NO:18] and
reverse 5' GCACATCTAGATCAGTGGTGGTGGTGGTGGTGGACCGGCCCCAGGA GTGG 3' [SEQ ID NO:19]

The PCR product of 985 bp obtained from amplification of HELA2 (testisin) from pBluescriptHELA2(S) as template was ligated into pGEM-T (Easy) vector (Promega). Digestion of this shuttle construct with NotI released a 1025 bp fragment which was ligated into pcDNA3 generating the short isoform expression construct pcDNA3-Test(S-C) (Figure 8). PCR amplification of the long isoform template gave a 991 bp product which was ligated into pGEM-T (easy) vector. NotI digestion of the shuttle construct released a 1031 bp fragment which was ligated into pcDNA3 giving pcDNA3-Test(L-C) (Figure 8).

Soluble testisin (1-295)-His6 in which the membrane anchoring sequence is deleted and the protein is carboxy-His6 tagged is to be obtained by PCR amplification of HELA2 (testisin) from pBluescriptHELA2(L) using the primers:
forward: 5' GCACAGCGGCCGCGAGGCCATGGGCGCGCGC 3' [SEQ ID NO:20] and
reverse: 5' GCACAGCGGCCGCTCAGTGGTGGTGGTGGTGGTGCCAGGAGGGGTC TGGCTG 3' [SEQ ID NO:21].
The PCR product will be digested with NotI and ligated into pcDNA3 generating the long isoform expression construct pcDNA3-Test(1-295)L-C (Figure 8).

(B) Expression and cellular localisation of HELA2 (testisin)

Each of the expression constructs is transiently transfected into a eukaryotic cell line (eg. HeLa, CHO or COS cells) by electroporation. Expression is confirmed by Northern blot and immunoblot. The His6 tag is a small, uncharged tag which reportedly does not interfere with cellular membrane interactions and is able to be detected with anti-His6 antibodies. HELA2 (testisin) cellular localisation is analysed by immunofluorescence using antibodies directed against the His6 tag and stained cells examined by confocal microscopy. Mock transfected cells is monitored as one of the controls in these experiments. Cells are examined under non-permeablised and permeabilised conditions to investigate intracellular and cell surface expression of HELA2 (testisin) tagged proteins. Possible release of HELA2 (testisin) into the supernatant is monitored by immunoblotting of conditioned media. Association of HELA2 (testisin) with a particular cellular compartment is confirmed by cellular fractionation studies. Stable transfectants of the full length and truncated tagged HELA2 (testisin) is generated by selection in G418. Recombinant HELA2 (testisin) is purified from these stable transfectants using a metal affinity resin (eg. Qiagen or Clontech) for assay of its bioactivity and efficacy as a therapeutic reagent.

EXAMPLE 7

HELA2 (TESTISIN) IS SPECIFICALLY EXPRESSED IN THE NORMAL TESTIS, AND IS ASSOCIATED WITH SPERM DEVELOPMENT

(A) Normal Tissue Blot

Dot blot analysis of PolyA+ RNA from 50 normal tissue specimens (strandardised to 8 different housekeeping genes) (Clontech) was performed using a 32P-labelled HELA2 (testisin) probe. Hybridization of the radiolabelled probe was in ExpressHyb solution (Clontech) at 65o. The blots were washed to a final stringency of 0.1xSSC/0.5% w/v SDS. High level expression of HELA2 (testisin) was found only in the testis as shown by the histogram plot of the Signal Intensity in Figure 9. In contrast, probing of the same blot with BCON3 showed ubiquitous expression of BCON3 mRNA in a variety of tissues (Figure 9).

(B) Multiple Tissue Northern Blot

Northern blots displaying polyA+ mRNA from 16 different normal tissues (Clontech) were hybridised at 65°C in ExpressHyb solution using a 400bp SacII/EcoRI 32P-labelled HELA2 probe for 3h and then washed to a final stringency of 0.1xSSC/0.1%SDS at 60°C. After a 5h exposure, a strong band was observed only in the lane containing testis mRNA, demonstrating the specificity of HELA2 (testisin) expression for the testis (Figure 10A). Prolonged exposure (4.5 days) of the blot revealed a very low level of HELA2 (testisin) mRNA expression in the prostate, lung and pancreas only. In contrast to HELA2, BCON3 is expressed in mRNA from most tissues present on the blot (Figure 10B).

(C) HELA2 (testisin) is Expressed in Sperm Cells, Demonstrating its Germ Cell Origin

To determine whether HELA2 (testisin) expression is associated with germ cells of the testis, ejaculate specimens from normal fertile males were compared with those of post-vasectomy males by RT-PCR analysis using HELA2 (testisin) specific primers. Sperm is the primary product from the testis that is found in ejaculate; other components of the ejaculate are derived from the prostate.

First strand cDNA was reverse transcribed from total RNA which has been isolated from frozen or fresh ejaculate specimens. PCR was performed on the cDNA templates using the primers:
forward: 5' CTGACTTCCATGCCATCCTT 3' [SEQ ID NO:22] and
reverse: 5' GCTCACGACTCCAATCTGAT 3' [SEQ ID NO:23].
As shown in Figure 11, strong signals of the expected size of 464 bp were detected in ejaculate from normal males (Patients #23 and #31), while no HELA2 (testisin) was detected in Patient #153 (post-vasectomy). Patent #90 (post-vasectomy) showed a low level of amplification product which may reflect a small amount of residual sperm in the seminiferous tubules. PCR using primers specific for (2-macroglobulin was performed on the same samples as a control for the presence of approximately equal amounts of cDNA in each sample.

(D) HELA2 (testisin) is Expressed in Immature Germ Cells of the Testis

In situ hybridization was performed on paraffin-embedded specimens of rat testis tissue using DIG- labelled HELA2 (testisin) RNA probes (T3 and T7 generated transcripts containing nucleotides 1-423 of HELA2 cDNA). The results using the antisense RNA probe showed strong positive staining near the basal lamina of the seminiferous tubules in the region associated with spermatocytes and spermatogonia (Figure 12, see arrows). HELA2 (testisin) mRNA expression did not appear to be associated with Leydig cells and the pattern was not typical for Sertoli cell staining. The presence of HELA2 (testisin) mRNA in these cells indicates a role for HELA2 (testisin) in germ cell maturation and sperm development.

EXAMPLE 8
HELA2 (TESTISIN) EXPRESSION IS ASSOCIATED WITH TUMOURS IN NON-TESTIS CELL-TYPES

The tissue and cell-type distribution of testisin mRNA transcripts in tumours were determined by Northern hybridization analyses of RNA extracted from in vitro cultured tumour cells lines derived from different cancerous tissues. HELA2 (testisin) was detected in the HeLa ovarian carcinoma, the U937 lymphoma, and the melanoma cell line 253-3D. HELA2 (testisin) is also associated with cDNA libraries derived from tumours of the colon, pancreas, prostate and ovary (NCBI-EST Database). The presence of HELA2 (testisin) in tumours where it is not expressed normally indicates that it likely plays a role in tumourigenesis in several cell-types.

EXAMPLE 9
THE HELA2 (TESTISIN) GENE IS LOCATED ON HUMAN CHROMOSOME 16p13.3

The genetic location of testisin was mapped to the short arm of chromosome 16 at 16p13.3 by fluorescence in-situ hybridization to normal metaphase chromosomes (Figure 13A). Screening of a chromosome 16 hybrid panel then sub-localised HELA2 (testisin) to the cosmid 406D6 which has been mapped to this region (Sood, R. et al (1997)Genomics 42: 83-95; Doggett, N.A. et al. (1995) Nature 377 (Suppl.):335-365. The cosmid lies between the markers

D16S246 and D16S468 and the gene is located just centromeric to D16S246 (Figure 13B). This region of the human genome is associated with high genetic instability and telomeric rearrangements underlie a variety of common human genetic disorders. Testisin is sandwiched between the human disease genes PKD1 (polycystic kidney disease) and tuberous sclerosis (TSC2) on the on side, and MEF (familial mediterranean fever) and Rubenstein-Taybi syndrome (RSTS) on the other side as diagrammed in Figure 13B.

EXAMPLE 10
HELA2 (TESTISIN) mRNA AND PROTEIN EXPRESSION IS ABSENT IN TESTICULAR GERM CELL TUMOURS

To determine whether HELA2 (testisin) may play a role in testicular tumourigenesis, HELA2 (testisin) mRNA expression in normal testes and testicular tumour tissue obtained from 4 patients diagnosed with seminoma were compared by Northern blot analysis. HELA2 (testisin) mRNA was detected in normal testes from all four patients but was not detectable in the corresponding tumours (Figure 14A). This data indicates a tumour suppressor role for HELA2 (testisin) in testicular germ cell tumours.

Expression of HELA2 (testisin) protein in testicular tissue was examined by immunohistochemistry. Paraffin-embedded tissue sections were fixed, treated, blocked, incubated with anti-peptide antibodies (1:10 dilution) and bound antibody detected with the Vectastain Universal Elite ABC kit (Vector Laboratories). Negative controls were performed in the absence of antibody. Strong staining of HELA2 (testisin) was detected in the germ cells of normal testis (N) but was absent in the adjacent tumour tissue (T) (for example, see Figure 14B), providing further evidence of a tumour suppressor role for HELA2 (testisin) in testicular germ cell tumours.

EXAMPLE 11
GENOMIC ORGANISATION OF THE HELA2 (TESTISIN) GENE

The HELA2 (testisin) gene is further characterised by determination of its genomic

organisation. Intron-extron boundaries and most of the DNA sequence of the HELA2 (testisin) gene was determined from cosmid DNA by DNA sequencing. A genomic map of HELA2 (testisin) is given in Figure 15. The intron/exon boundaries are highly conserved relative to prostasin, although the sizes of the introns show considerable variation. The genomic DNA sequence with introns in lower case and exons in upper case is shown in Figure 16 and in SEQ ID NO 25. DNA sequence analysis is being performed on RNA from tumour tissues to ascertain the predicted function of HELA2 (testisin) as a tumour suppressor.

EXAMPLE 12

THE HELA2 (TESTISIN) SHORT AND LONG ISOFORMS ARE GENERATED BY ALTERNATIVE mRNA SPLICING

Two isoforms of HELA2 (testisin) were identified which differ by an insertion of 2 amino acids (Tyr-Ser) between the catalytic His and Asp residues. These constitute the long (L) and short (S) isoforms. At the DNA level there is a corresponding insertion of 6 nucleotides which generates a Sfc1 restriction enzyme site. PCR amplification from single strand cDNA generated from HeLa cell total RNA followed by DNA sequence analysis of the amplified product demonstrated that the two isoforms are generated through the use of two alternative mRNA splice sites. The DNA sequence for the intron and the flanking exons are shown in Figure 17. The resulting insertion of amino acids YS occurs 4 amino acids after the catalytic His residue of HELA2 (testisin). Preliminary molecular modelling shows the presence of this insertion is likely to alter the catalytic activity and/or specificity of HELA2 (testisin) for its substrates.

EXAMPLE 13

MUTATION ANALYSIS-HELA2 (TESTISIN) AS A TUMOUR SUPPRESSOR

Intronic DNA sequence information generated above (see Example 11) is used to generate primers to amplify HELA2 (testisin) exons for SSCP analyses. Genomic DNA isolated from seminomas and corresponding normal testis as well as genomic DNA from wild-type and affected seminoma family members are analysed by SSCP for altered expression patterns indicative of genetic mutations. Evidence of genetic mutations are also being determined by

DNA sequence analysis.

EXAMPLE 14
HOMOLOGUES OF HUMAN HELA2 (TESTISIN) ARE PRESENT IN OTHER SPECIES

Southern blot analysis of genomic DNA isolated from a range of species using a HELA2 (testisin) cDNA probe shows that homologues of HELA2 (testisin) are present in hamster, mouse, marmoset and monkey. The mouse homologue of HELA2 (testisin) was identified and obtained as an EST clone. The cDNA sequence and corresponding amino acid sequence of mouse HELA2 (testisin) was determined (Figure 18) and is given in SEQ ID NO 27. The mouse cDNA encodes a protein which contains the catalytic triad of His, Asp and Ser (circles) and 10 cysteine residues (small boxes), and an activation site (triangle) as found in HELA2 (testisin). The hydrophilicity plot shows the presence of a hydrophobic sequence at the carboxy terminus suggesting the presence of a putative membrane anchor. Comparison of the mouse and human sequences show 68.1% homology at the cDNA level and 69.1% homology at the amino acid level.

EXAMPLE 15
HELA2 (TESTISIN) IS PART OF A CLUSTER OF HOMOLOGOUS GENES ON CHROMOSOME 16p13.3

Analysis of DNA sequences released to NCBI databases reveals the presence of homologues of HELA2 (testisin) in a cluster on Chromosome 16p13.3. Figure 19 shows the positions of these genes, designated SP001LA, SP002LA, SP003LA, and SP004LA, relative to HELA2 (testisin) and the respective cosmids (Sood, R. et al (1997) Genomics 42: 83-95) in which they are located. Figure 20A, 20B and 20C show the partial cDNA and deduced amino acid sequences of SP001LA, SP002LA, and SP003LA respectively. Each cDNA encodes a protein which contains the catalytic triad of His, Asp and Ser (circles) and 10 cysteine residues (small boxes), and an activation site (triangle) as found in HELA2 (testisin). Comparisons of the cDNA and amino acid sequences from the heavy chain region through to the poly A tail gives

the % identity with HELA2 (testisin) as follows:

	cDNA	Protein
SP001LA	34.8%	47.3%
SP002LA	41.0%	47.1%
SP003LA	40.3%	51.3%

Each of the serine proteinases encoded by these genes show that they have carboxy terminal extensions, and SP002LA is the only one with a hydrophobic carboxy terminal tail indicative of a membrane anchored protein. Identification of an expressed sequence tag (EST) from a human testis cDNA library demonstrates that this gene is expressed in the testis, like HELA2 (testisin). The location of this serine proteinase cluster on chromosome 16p13.3 flanking HELA2 (testisin) suggests that these serine proteinases are also involved, like HELA2(testisin), in sperm maturation and development. Thus they may constitute a proteolytic cascade which is essential for these processes. Loss or mutation of these genes may lead to testicular germ cell tumours and to other testicular abnormalities, such as infertility.

EXAMPLE 16
ATC2 SERINE PROTEINASE

ATC2 was isolated from the cDNA of PAI-2 expressing HeLa cells following treatment with TNF and cycloheximide. A partial DNA sequence for ATC2 cDNA has been obtained which encompasses the sequence encoding the serine proteinase catalytic region. Additional clones extending to both 5' and 3' directions have been obtained. The available nucleic acid sequence of ATC2 cDNA and its deduced amino acid sequence shows that it is a member of the serine proteinase family with homology to hepsin, prostasin, and acrosin. It thus belongs to the same family as HELA2. The catalytic region includes the His, Asp and Ser conserved motifs. Preliminary Northern blot experiments have failed to detect ATC2 mRNA in total RNA isolated from resting HeLa cells, indicating it is not expressed in abundance in these cells, which may therefore be tightly regulated. As ATC2 was isolated from cells following treatment with TNF and cycloheximide, its expression may be induced by these agents in HeLa cells. These data

have potential significance for a role for ATC2 in apoptosis and cell death. ATC2 may be intracellular, extracellular or found on the cell surface and is likely to be involved in regulating cell functions. Thus ATC2 may have potential significance in the treatment of cancer and diseases involving dysregulation of cell growth and survival. The nucleotide and corresponding amino acid sequence of ATC2 is shown in SEQ ID NOs: 7 and 8, respectively.

EXAMPLE 16
BCON3

The deduced amino acid sequence of BCON3 (SEQ ID NO:10) reveals that it is novel. At both the DNA and protein level, BCON3 shows homology to members of the kinase family of proteins. Although it cannot be classified as a member of any particular sub-family of kinases, alignments of the BCON3 protein with the conserved domains of thymidine kinases and tyrosine and serine/threonine protein kinases indicates possible ATP/GTP binding and phosphate transfer regions. Thus, it may be the first member of a new family of kinases. Analysis of the translation product using hydrophobicity plots and the Prosite protein analysis algorithms indicates BCON3 may lack an N-terminal signal sequence (that is, it is likely to encode an intracellular protein) and it possesses a nuclear localization signal. BCON3 mRNA is approximately 2300 nucleotides in length. cDNA sequence (SEQ ID NO:9) has been obtained covering about 95% of the transcript and including the 3' poly A tail. BCON3 mRNA is expressed in most normal tissues as demonstrated by dot blot analysis of 50 normal tissue specimens (standardised to 8 different housekeeping genes) (Clontech). (Figure 9). Analysis of BCON3 mRNA expression using a multiple tissue Northern blot displaying polyA+ mRNA from 16 different normal tissues (Clontech) shows that BCON3 is expressed in most tissues (Figure 10B). Expression by in vitro transcription/translation expression using a partial BCON3 cDNA fragment shows BCON3 encodes a protein. Two major transcription/translation products are detected, one of 51kDa, the size predicted from the open reading frame, and a second product of about 43kDa, which may represent a partial translation product (Figure 21).

Those skilled in the art will appreciate that the invention described herein is susceptible to

variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT
(US only): ANTALIS Toni Marie and HOOPER John David
(Other than US): AMRAD OPERATIONS PTY LTD

(ii) TITLE OF INVENTION: NOVEL MOLECULES

(iii) NUMBER OF SEQUENCES: 24

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: International PCT Application
(B) FILING DATE: 13-FEB-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PO5101/97
(B) FILING DATE: 13-FEB-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PP0422/97
(B) FILING DATE: 18-NOV-1997
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: HUGHES, DR E JOHN L
(C) REFERENCE/DOCKET NUMBER: EJH/AF

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +61 3 9254 2777
(B) TELEFAX: +61 3 9254 2770
(C) TELEX: AA 31787

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACAGAATTCT GGGTIGTIAC IGCIGCICAY TG 32

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1094 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACAGAATTCA XIGGICCICC IC/GT/AXTCICC 29

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1094 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 17..965

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
CGCGGGAGAG GAGGCC ATG GGC GCG CGC GGG GCG CTG CTG CTG GCG CTG       49
                  Met Gly Ala Arg Gly Ala Leu Leu Leu Ala Leu
                   1               5                  10

CTG CTG GCT CGG GCT GGA CTC AGG AAG CCG GAG TCG CAG GAG GCG GCG      97
Leu Leu Ala Arg Ala Gly Leu Arg Lys Pro Glu Ser Gln Glu Ala Ala
            15                  20                  25

CCG TTA TCA GGA CCA TGC GGC CGA CGG GTC ATC ACG TCG CGC ATC GTG     145
Pro Leu Ser Gly Pro Cys Gly Arg Arg Val Ile Thr Ser Arg Ile Val
        30                  35                  40

GGT GGA GAG GAC GCC GAA CTC GGG CGT TGG CCG TGG CAG GGG AGC CTG     193
Gly Gly Glu Asp Ala Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser Leu
    45                  50                  55

CGC CTG TGG GAT TCC CAC GTA TGC GGA GTG AGC CTG CTC AGC CAC CGC     241
Arg Leu Trp Asp Ser His Val Cys Gly Val Ser Leu Leu Ser His Arg
 60                  65                  70                  75

TGG GCA CTC ACG GCG GCG CAC TGC TTT GAA ACT GAC CTT AGT GAT CCC     289
Trp Ala Leu Thr Ala Ala His Cys Phe Glu Thr Asp Leu Ser Asp Pro
                 80                  85                  90

TCC GGG TGG ATG GTC CAG TTT GGC CAG CTG ACT TCC ATG CCA TCC TTC     337
Ser Gly Trp Met Val Gln Phe Gly Gln Leu Thr Ser Met Pro Ser Phe
            95                 100                 105

TGG AGC CTG CAG GCC TAC TAC ACC CGT TAC TTC GTA TCG AAT ATC TAT     385
Trp Ser Leu Gln Ala Tyr Tyr Thr Arg Tyr Phe Val Ser Asn Ile Tyr
        110                 115                 120

CTG AGC CCT CGC TAC CTG GGG AAT TCA CCC TAT GAC ATT GCC TTG GTG     433
Leu Ser Pro Arg Tyr Leu Gly Asn Ser Pro Tyr Asp Ile Ala Leu Val
    125                 130                 135

AAG CTG TCT GCA CCT GTC ACC TAC ACT AAA CAC ATC CAG CCC ATC TGT     481
Lys Leu Ser Ala Pro Val Thr Tyr Thr Lys His Ile Gln Pro Ile Cys
140                 145                 150                 155

CTC CAG GCC TCC ACA TTT GAG TTT GAG AAC CGG ACA GAC TGC TGG GTG     529
Leu Gln Ala Ser Thr Phe Glu Phe Glu Asn Arg Thr Asp Cys Trp Val
                160                 165                 170
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ACT GGC TGG GGG TAC ATC AAA GAG GAT GAG GCA CTG CCA TCT CCC CAC       577
Thr Gly Trp Gly Tyr Ile Lys Glu Asp Glu Ala Leu Pro Ser Pro His
            175                 180                 185

ACC CTC CAG GAA GTT CAG GTC GCC ATC ATA AAC AAC TCT ATG TGC AAC       625
Thr Leu Gln Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met Cys Asn
        190                 195                 200

CAC CTC TTC CTC AAG TAC AGT TTC CGC AAG GAC ATC TTT GGA GAC ATG       673
His Leu Phe Leu Lys Tyr Ser Phe Arg Lys Asp Ile Phe Gly Asp Met
    205                 210                 215

GTT TGT GCT GGC AAT GCC CAA GGC GGG AAG GAT GCC TGC TTC GGT GAC       721
Val Cys Ala Gly Asn Ala Gln Gly Gly Lys Asp Ala Cys Phe Gly Asp
220                 225                 230                 235

TCA GGT GGA CCC TTG GCC TGT AAC AAG GAT GGA CTG TGG TAT CAG ATT       769
Ser Gly Gly Pro Leu Ala Cys Asn Lys Asp Gly Leu Trp Tyr Gln Ile
                240                 245                 250

GGA GTC GTG AGC TGG GGA GTG GGC TGT GGT CGG CCC AAT CGG CCC GGT       817
Gly Val Val Ser Trp Gly Val Gly Cys Gly Arg Pro Asn Arg Pro Gly
            255                 260                 265

GTC TAC ACC AAT ATC AGC CAC CAC TTT GAG TGG ATC CAG AAG CTG ATG       865
Val Tyr Thr Asn Ile Ser His His Phe Glu Trp Ile Gln Lys Leu Met
        270                 275                 280

GCC CAG AGT GGC ATG TCC CAG CCA GAC CCC TCC TGG CCG CTA CTC TTT       913
Ala Gln Ser Gly Met Ser Gln Pro Asp Pro Ser Trp Pro Leu Leu Phe
    285                 290                 295

TTC CCT CTT CTC TGG GCT CTC CCA CTC CTG GGG CCG GTC TGA               961
Phe Pro Leu Leu Trp Ala Leu Pro Leu Leu Gly Pro Val  *
300                 305                 310

GCCTACCTGA GCCCATGCAG CCTGGGGCCA CTGCCAAGTC AGGCCCTGGT TCTCTTCTGT    1015

CTTGTTTGGT AATAAACACA TTCCAGTTGA TGCCTTGCAG GGCATTTTTC AAAAAAAAAA    1075

AAAAAAAAAA AAAAAAAAA                                                  1094
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 313 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Gly Ala Arg Gly Ala Leu Leu Leu Ala Leu Leu Leu Ala Arg Ala
 1               5                  10                  15

Gly Leu Arg Lys Pro Glu Ser Gln Glu Ala Ala Pro Leu Ser Gly Pro
            20                  25                  30

Cys Gly Arg Arg Val Ile Thr Ser Arg Ile Val Gly Gly Glu Asp Ala
        35                  40                  45

Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser Leu Arg Leu Trp Asp Ser
    50                  55                  60

His Val Cys Gly Val Ser Leu Leu Ser His Arg Trp Ala Leu Thr Ala
65                  70                  75                  80

Ala His Cys Phe Glu Thr Asp Leu Ser Asp Pro Ser Gly Trp Met Val
                85                  90                  95

Gln Phe Gly Gln Leu Thr Ser Met Pro Ser Phe Trp Ser Leu Gln Ala
            100                 105                 110

Tyr Tyr Thr Arg Tyr Phe Val Ser Asn Ile Tyr Leu Ser Pro Arg Tyr
        115                 120                 125

Leu Gly Asn Ser Pro Tyr Asp Ile Ala Leu Val Lys Leu Ser Ala Pro
    130                 135                 140

Val Thr Tyr Thr Lys His Ile Gln Pro Ile Cys Leu Gln Ala Ser Thr
145                 150                 155                 160

Phe Glu Phe Glu Asn Arg Thr Asp Cys Trp Val Thr Gly Trp Gly Tyr
                165                 170                 175

Ile Lys Glu Asp Glu Ala Leu Pro Ser Pro His Thr Leu Gln Glu Val
            180                 185                 190
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Gln Val Ala Ile Ile Asn Asn Ser Met Cys Asn His Leu Phe Leu Lys
        195                 200                 205

Tyr Ser Phe Arg Lys Asp Ile Phe Gly Asp Met Val Cys Ala Gly Asn
    210                 215                 220

Ala Gln Gly Gly Lys Asp Ala Cys Phe Gly Asp Ser Gly Gly Pro Leu
225                 230                 235                 240

Ala Cys Asn Lys Asp Gly Leu Trp Tyr Gln Ile Gly Val Val Ser Trp
                245                 250                 255

Gly Val Gly Cys Gly Arg Pro Asn Arg Pro Gly Val Tyr Thr Asn Ile
            260                 265                 270

Ser His His Phe Glu Trp Ile Gln Lys Leu Met Ala Gln Ser Gly Met
        275                 280                 285

Ser Gln Pro Asp Pro Ser Trp Pro Leu Leu Phe Phe Pro Leu Leu Trp
    290                 295                 300

Ala Leu Pro Leu Leu Gly Pro Val  *
305                 310
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1100 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 17..961

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CGCGGGAGAG GAGGCC ATG GGC GCG CGC GGG GCG CTG CTG CTG GCG CTG      49
                  Met Gly Ala Arg Gly Ala Leu Leu Leu Ala Leu
```

```
                    1                   5                   10

CTG CTG GCT CGG GCT GGA CTC AGG AAG CCG GAG TCG CAG GAG GCG GCG        97
Leu Leu Ala Arg Ala Gly Leu Arg Lys Pro Glu Ser Gln Glu Ala Ala
                15                  20                  25

CCG TTA TCA GGA CCA TGC GGC CGA CGG GTC ATC ACG TCG CGC ATC GTG       145
Pro Leu Ser Gly Pro Cys Gly Arg Arg Val Ile Thr Ser Arg Ile Val
            30                  35                  40

GGT GGA GAG GAC GCC GAA CTC GGG CGT TGG CCG TGG CAG GGG AGC CTG       193
Gly Gly Glu Asp Ala Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser Leu
        45                  50                  55

CGC CTG TGG GAT TCC CAC GTA TGC GGA GTG AGC CTG CTC AGC CAC CGC       241
Arg Leu Trp Asp Ser His Val Cys Gly Val Ser Leu Leu Ser His Arg
 60                  65                  70                  75

TGG GCA CTC ACG GCG GCG CAC TGC TTT GAA ACC TAT AGT GAC CTT AGT       289
Trp Ala Leu Thr Ala Ala His Cys Phe Glu Thr Tyr Ser Asp Leu Ser
                    80                  85                  90

GAT CCC TCC GGG TGG ATG GTC CAG TTT GGC CAG CTG ACT TCC ATG CCA       337
Asp Pro Ser Gly Trp Met Val Gln Phe Gly Gln Leu Thr Ser Met Pro
                95                 100                 105

TCC TTC TGG AGC CTG CAG GCC TAC TAC ACC CGT TAC TTC GTA TCG AAT       385
Ser Phe Trp Ser Leu Gln Ala Tyr Tyr Thr Arg Tyr Phe Val Ser Asn
           110                 115                 120

ATC TAT CTG AGC CCT CGC TAC CTG GGG AAT TCA CCC TAT GAC ATT GCC       433
Ile Tyr Leu Ser Pro Arg Tyr Leu Gly Asn Ser Pro Tyr Asp Ile Ala
       125                 130                 135

TTG GTG AAG CTG TCT GCA CCT GTC ACC TAC ACT AAA CAC ATC CAG CCC       481
Leu Val Lys Leu Ser Ala Pro Val Thr Tyr Thr Lys His Ile Gln Pro
140                 145                 150                 155

ATC TGT CTC CAG GCC TCC ACA TTT GAG TTT GAG AAC CGG ACA GAC TGC       529
Ile Cys Leu Gln Ala Ser Thr Phe Glu Phe Glu Asn Arg Thr Asp Cys
                160                 165                 170

TGG GTG ACT GGC TGG GGG TAC ATC AAA GAG GAT GAG GCA CTG CCA TCT       577
Trp Val Thr Gly Trp Gly Tyr Ile Lys Glu Asp Glu Ala Leu Pro Ser
                175                 180                 185
```

CCC CAC ACC CTC CAG GAA GTT CAG GTC GCC ATC ATA AAC AAC TCT ATG 625
Pro His Thr Leu Gln Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met
190 195 200

TGC AAC CAC CTC TTC CTC AAG TAC AGT TTC CGC AAG GAC ATC TTT GGA 673
Cys Asn His Leu Phe Leu Lys Tyr Ser Phe Arg Lys Asp Ile Phe Gly
205 210 215

GAC ATG GTT TGT GCT GGC AAT GCC CAA GGC GGG AAG GAT GCC TGC TTC 721
Asp Met Val Cys Ala Gly Asn Ala Gln Gly Gly Lys Asp Ala Cys Phe
220 225 230 235

GGT GAC TCA GGT GGA CCC TTG GCC TGT AAC AAG GAT GGA CTG TGG TAT 769
Gly Asp Ser Gly Gly Pro Leu Ala Cys Asn Lys Asp Gly Leu Trp Tyr
240 245 250

CAG ATT GGA GTC GTG AGC TGG GGA GTG GGC TGT GGT CGG CCC AAT CGG 817
Gln Ile Gly Val Val Ser Trp Gly Val Gly Cys Gly Arg Pro Asn Arg
255 260 265

CCC GGT GTC TAC ACC AAT ATC AGC CAC CAC TTT GAG TGG ATC CAG AAG 865
Pro Gly Val Tyr Thr Asn Ile Ser His His Phe Glu Trp Ile Gln Lys
270 275 280

CTG ATG GCC CAG AGT GGC ATG TCC CAG CCA GAC CCC TCC TGG CCG CTA 913
Leu Met Ala Gln Ser Gly Met Ser Gln Pro Asp Pro Ser Trp Pro Leu
285 290 295

CTC TTT TTC CCT CTT CTC TGG GCT CTC CCA CTC CTG GGG CCG GTC TGAGCCTACC
968
Leu Phe Phe Pro Leu Leu Trp Ala Leu Pro Leu Leu Gly Pro Val
300 305 310 315

TGAGCCCATG CAGCCTGGGG CCACTGCCAA GTCAGGCCCT GGTTCTCTTC TGTCTTGTTT 1028

GGTAATAAAC ACATTCCAGT TGATGCCTTG CAGGGCATTT TTCAAAAAAA AAAAAAAAAA 1088

AAAAAAAAAA AA 1100

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 314 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gly Ala Arg Gly Ala Leu Leu Leu Ala Leu Leu Leu Ala Arg Ala
1 5 10 15

Gly Leu Arg Lys Pro Glu Ser Gln Glu Ala Ala Pro Leu Ser Gly Pro
20 25 30

Cys Gly Arg Arg Val Ile Thr Ser Arg Ile Val Gly Gly Glu Asp Ala
35 40 45

Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser Leu Arg Leu Trp Asp Ser
50 55 60

His Val Cys Gly Val Ser Leu Leu Ser His Arg Trp Ala Leu Thr Ala
65 70 75 80

Ala His Cys Phe Glu Thr Tyr Ser Asp Leu Ser Asp Pro Ser Gly Trp
85 90 95

Met Val Gln Phe Gly Gln Leu Thr Ser Met Pro Ser Phe Trp Ser Leu
100 105 110

Gln Ala Tyr Tyr Thr Arg Tyr Phe Val Ser Asn Ile Tyr Leu Ser Pro
115 120 125

Arg Tyr Leu Gly Asn Ser Pro Tyr Asp Ile Ala Leu Val Lys Leu Ser
130 135 140

Ala Pro Val Thr Tyr Thr Lys His Ile Gln Pro Ile Cys Leu Gln Ala
145 150 155 160

Ser Thr Phe Glu Phe Glu Asn Arg Thr Asp Cys Trp Val Thr Gly Trp
165 170 175

Gly Tyr Ile Lys Glu Asp Glu Ala Leu Pro Ser Pro His Thr Leu Gln
180 185 190

Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met Cys Asn His Leu Phe
195 200 205

Leu Lys Tyr Ser Phe Arg Lys Asp Ile Phe Gly Asp Met Val Cys Ala
    210   215   220

Gly Asn Ala Gln Gly Gly Lys Asp Ala Cys Phe Gly Asp Ser Gly Gly
225   230   235   240

Pro Leu Ala Cys Asn Lys Asp Gly Leu Trp Tyr Gln Ile Gly Val Val
                245   250   255

Ser Trp Gly Val Gly Cys Gly Arg Pro Asn Arg Pro Gly Val Tyr Thr
            260   265   270

Asn Ile Ser His His Phe Glu Trp Ile Gln Lys Leu Met Ala Gln Ser
        275   280   285

Gly Met Ser Gln Pro Asp Pro Ser Trp Pro Leu Leu Phe Phe Pro Leu
    290   295   300

Leu Trp Ala Leu Pro Leu Leu Gly Pro Val
305   310

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 799 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 24..799

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AGTTCAGATG AATGGGACTG TGA GAA CCA TCT GTG ACC AAA TTG ATA CAG 50
                          Glu Pro Ser Val Thr Lys Leu Ile Gln
                            1   5

GAA CAG GAG AAA GAG CCG CGG TGG CTG ACA TTA CAC TCC AAC TGG GAG 98

Glu Gln Glu Lys Glu Pro Arg Trp Leu Thr Leu His Ser Asn Trp Glu
10 15 20 25

AGC CTC AAT GGG ACC ACT TTA CAT GAA CTT GTA GTA AAT GGG CAG TCT 146
Ser Leu Asn Gly Thr Thr Leu His Glu Leu Val Val Asn Gly Gln Ser
30 35 40

TGT GAG AGC AGA AGT AAA ATT TCT CTT CTG TGT ACT AAA CAA GAC TGT 194
Cys Glu Ser Arg Ser Lys Ile Ser Leu Leu Cys Thr Lys Gln Asp Cys
45 50 55

GGG CGC CGC CCT GCT GCC CGA ATG AAC AAA AGG ATC CTT GGA GGT CGG 242
Gly Arg Arg Pro Ala Ala Arg Met Asn Lys Arg Ile Leu Gly Gly Arg
60 65 70

ACG AGT CGC CCT GGA AGG TGG CCA TGG CAG TGT TCT CTG CAG AGT GAA 290
Thr Ser Arg Pro Gly Arg Trp Pro Trp Gln Cys Ser Leu Gln Ser Glu
75 80 85

CCC AGT GGA CAT ATC TGT GGC TGT GTC CTC ATT GCC AAG AAG TGG GTT 338
Pro Ser Gly His Ile Cys Gly Cys Val Leu Ile Ala Lys Lys Trp Val
90 95 100 105

GTG ACA GTT GCC CAC TGC TTC GAG GGG AGA GAG AAT GCT GCA GTT TGG 386
Val Thr Val Ala His Cys Phe Glu Gly Arg Glu Asn Ala Ala Val Trp
110 115 120

AAA GTG GTG CTT GGC ATC AAC AAT CTA GAC CAT CCA TCA GTG TTC ATG 434
Lys Val Val Leu Gly Ile Asn Asn Leu Asp His Pro Ser Val Phe Met
125 130 135

CAG ACA CGC TTT GTG AGG ACC ATC ATC CTG CAT CCC CGC TAC AGT CGA 482
Gln Thr Arg Phe Val Arg Thr Ile Ile Leu His Pro Arg Tyr Ser Arg
140 145 150

GCA GTG GTG GAC TAT GAC ATC AGC ATC GTT GAG CTG AGT GAA GAC ATC 530
Ala Val Val Asp Tyr Asp Ile Ser Ile Val Glu Leu Ser Glu Asp Ile
155 160 165

AGT GAG ACT GGC TAC GTC CGG CCT GTC TGC TTG CCC AAC CCG GAG CAG 578
Ser Glu Thr Gly Tyr Val Arg Pro Val Cys Leu Pro Asn Pro Glu Gln
170 175 180 185

TGG CTA GAG CCT GAC ACG TAC TGC TAT ATC ACA GGC TGG GGC CAC ATG 626
Trp Leu Glu Pro Asp Thr Tyr Cys Tyr Ile Thr Gly Trp Gly His Met

```
                190                 195                 200

GGC AAT AAA ATG CCA TTT AAG CTG CAA GAG GGA GAG GTC CGC ATT ATT        674
Gly Asn Lys Met Pro Phe Lys Leu Gln Glu Gly Glu Val Arg Ile Ile
            205                 210                 215

TCT CTG GAA CAT TGT CAG TCC TAC TTT GAC ATG AAG ACC ATC ACC ACT        722
Ser Leu Glu His Cys Gln Ser Tyr Phe Asp Met Lys Thr Ile Thr Thr
        220                 225                 230

CGG ATG ATA TGT GCT GGC TAT GAG TCT GGC ACA GTT GAT TCA TGC ATG        770
Arg Met Ile Cys Ala Gly Tyr Glu Ser Gly Thr Val Asp Ser Cys Met
    235                 240                 245

GGT GAC TGG GGC GGT CCG TTG AAT TCT GT                                 799
Gly Asp Trp Gly Gly Pro Leu Asn Ser
250                 255
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 258 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.8:

```
Glu Pro Ser Val Thr Lys Leu Ile Gln Glu Gln Glu Lys Glu Pro Arg
 1               5                  10                  15

Trp Leu Thr Leu His Ser Asn Trp Glu Ser Leu Asn Gly Thr Thr Leu
            20                  25                  30

His Glu Leu Val Val Asn Gly Gln Ser Cys Glu Ser Arg Ser Lys Ile
        35                  40                  45

Ser Leu Leu Cys Thr Lys Gln Asp Cys Gly Arg Arg Pro Ala Ala Arg
    50                  55                  60

Met Asn Lys Arg Ile Leu Gly Gly Arg Thr Ser Arg Pro Gly Arg Trp
65                  70                  75                  80
```

```
Pro Trp Gln Cys Ser Leu Gln Ser Glu Pro Ser Gly His Ile Cys Gly
                 85                  90                  95

Cys Val Leu Ile Ala Lys Lys Trp Val Val Thr Val Ala His Cys Phe
            100                 105                 110

Glu Gly Arg Glu Asn Ala Ala Val Trp Lys Val Val Leu Gly Ile Asn
        115                 120                 125

Asn Leu Asp His Pro Ser Val Phe Met Gln Thr Arg Phe Val Arg Thr
    130                 135                 140

Ile Ile Leu His Pro Arg Tyr Ser Arg Ala Val Val Asp Tyr Asp Ile
145                 150                 155                 160

Ser Ile Val Glu Leu Ser Glu Asp Ile Ser Glu Thr Gly Tyr Val Arg
                165                 170                 175

Pro Val Cys Leu Pro Asn Pro Glu Gln Trp Leu Glu Pro Asp Thr Tyr
            180                 185                 190

Cys Tyr Ile Thr Gly Trp Gly His Met Gly Asn Lys Met Pro Phe Lys
        195                 200                 205

Leu Gln Glu Gly Glu Val Arg Ile Ile Ser Leu Glu His Cys Gln Ser
    210                 215                 220

Tyr Phe Asp Met Lys Thr Ile Thr Thr Arg Met Ile Cys Ala Gly Tyr
225                 230                 235                 240

Glu Ser Gly Thr Val Asp Ser Cys Met Gly Asp Trp Gly Gly Pro Leu
                245                 250                 255

Asn Ser
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2241 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 166..1773

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
ATTTAATACG ACTCACTATA GGGAATTTGG CCCTCGAGGA AGAATTCGGC ACGAGGCTGC      60

GGCGCACTGT GAGGGAGTCG CTGTGATCCG GGGCCCCGAA CCCGACTGGA GCTGAAGCGC     120

AGGCTGCGGG GCGCGGAGTC GGGAGGCCTG AGTGTTCCTT CCAGC ATG TCG GAG         174
                                                  Met Ser Glu
                                                   1

GGG GAG TCC CAG ACA GTA CTT AGC AGT GGC TCA GAC CCA AAG GTA GAA       222
Gly Glu Ser Gln Thr Val Leu Ser Ser Gly Ser Asp Pro Lys Val Glu
    5                  10                  15

TCT TCA TCT TCA GCT CCT GGC CTG ACA TCA GTG TCA CCT CCT GTG ACC       270
Ser Ser Ser Ser Ala Pro Gly Leu Thr Ser Val Ser Pro Pro Val Thr
 20                 25                  30                  35

TCC ACA ACC TCA GCT GCT TCC CCA GAG GAA GAA GAA GAA AGT GAA GAT       318
Ser Thr Thr Ser Ala Ala Ser Pro Glu Glu Glu Glu Glu Ser Glu Asp
                40                  45                  50

GAG TCT GAG ATT TTG GAA GAG TCG CCC TGT GGG CGC TGG CAG AAG AGG       366
Glu Ser Glu Ile Leu Glu Glu Ser Pro Cys Gly Arg Trp Gln Lys Arg
            55                  60                  65

CGA GAA GAG GTG AAT CAA CGG AAT GTA CCA GGT ATT GAC AGT GCA TAC       414
Arg Glu Glu Val Asn Gln Arg Asn Val Pro Gly Ile Asp Ser Ala Tyr
        70                  75                  80

CTG GCC ATG GAT ACA GAG GAA GGT GTA GAG GTT GTG TGG AAT GAG GTA       462
Leu Ala Met Asp Thr Glu Glu Gly Val Glu Val Val Trp Asn Glu Val
    85                  90                  95

CAG TTC TCT GAA CGC AAG AAC TAC AAG CTG CAG GAG GAA AAG GTT TGT       510
Gln Phe Ser Glu Arg Lys Asn Tyr Lys Leu Gln Glu Glu Lys Val Cys
100                 105                 110                 115

GCT GTG TTT GAT AAT TTG ATT CAA TTG GAG CAT CTT AAC ATT GTT AAG       558
```

Ala Val Phe Asp Asn Leu Ile Gln Leu Glu His Leu Asn Ile Val Lys
120 125 130

TTT CAC AAA TAT TGG GCT GAC ATT AAA GAG AAC AAG GCC AGG GTC ATT 606
Phe His Lys Tyr Trp Ala Asp Ile Lys Glu Asn Lys Ala Arg Val Ile
135 140 145

TTT ATC ACA GGA TAC ATG TCA TCT GGG AGT CTG AAG CAA TTT CTG AAG 654
Phe Ile Thr Gly Tyr Met Ser Ser Gly Ser Leu Lys Gln Phe Leu Lys
150 155 160

AAG ACC CAA AAG AAC CAC CAG ACG ATG AAT GAA AAG GCA TGG AAG CGT 702
Lys Thr Gln Lys Asn His Gln Thr Met Asn Glu Lys Ala Trp Lys Arg
165 170 175

TGG TGC ACA CAA ATC CTC TCT GCC CTA AGC TAC CTG CAC TCC TGT GAC 750
Trp Cys Thr Gln Ile Leu Ser Ala Leu Ser Tyr Leu His Ser Cys Asp
180 185 190 195

CCC CCC ATC ATC CAT GGG AAC CTG ACC TGT GAC ACC ATC TTC ATC CAG 798
Pro Pro Ile Ile His Gly Asn Leu Thr Cys Asp Thr Ile Phe Ile Gln
200 205 210

CAC AAC GGA CTC ATC AAG ATT GGC TCT GTG GCT CCT GAC ACT ATC AAC 846
His Asn Gly Leu Ile Lys Ile Gly Ser Val Ala Pro Asp Thr Ile Asn
215 220 225

AAT CAT GTG AAG ACT TGT CGA GAA GAG CAG AAG AAT CTA CAC TTC TTT 894
Asn His Val Lys Thr Cys Arg Glu Glu Gln Lys Asn Leu His Phe Phe
230 235 240

GCA CCA GAG TAT GGA GAA GTC ACT AAT GTG ACA ACA GCA GTG GAC ATC 942
Ala Pro Glu Tyr Gly Glu Val Thr Asn Val Thr Thr Ala Val Asp Ile
245 250 255

TAC TCC TTT GGC ATG TGT GCA CTG GGG ATG GCA GTG CTG GAG ATT CAG 990
Tyr Ser Phe Gly Met Cys Ala Leu Gly Met Ala Val Leu Glu Ile Gln
260 265 270 275

GGC AAT GGA GAG TCC TCA TAT GTG CCA CAG GAA GCC ATC AGC AGT GCC 1038
Gly Asn Gly Glu Ser Ser Tyr Val Pro Gln Glu Ala Ile Ser Ser Ala
280 285 290

ATC CAG CTT CTA GAA GAC CCA TTA CAG AGG GAG TTC ATT CAA AAG TGC 1086
Ile Gln Leu Leu Glu Asp Pro Leu Gln Arg Glu Phe Ile Gln Lys Cys

295 300 305

CTG CAG TCT GAG CCT GCT CGC AGA CCA ACA GCC AGA GAA CTT CTG TTC 1134
Leu Gln Ser Glu Pro Ala Arg Arg Pro Thr Ala Arg Glu Leu Leu Phe
310 315 320

CAC CCA GCA TTG TTT GAA GTG CCC TCG CTC AAA CTC CTT GCG GCC CAC 1182
His Pro Ala Leu Phe Glu Val Pro Ser Leu Lys Leu Leu Ala Ala His
325 330 335

TGC ATT GTG GGA CAC CAA CAC ATG ATC CCA GAG AAC GCT CTA GAG GAG 1230
Cys Ile Val Gly His Gln His Met Ile Pro Glu Asn Ala Leu Glu Glu
340 345 350 355

ATC ACC AAA AAC ATG GAT ACT AGT GCC GTA CTG GCT GAA ATC CCT GCA 1278
Ile Thr Lys Asn Met Asp Thr Ser Ala Val Leu Ala Glu Ile Pro Ala
360 365 370

GGA CCA GGA AGA GAA CCA GTT CAG ACT TTG TAC TCT CAG TCA CCA GCT 1326
Gly Pro Gly Arg Glu Pro Val Gln Thr Leu Tyr Ser Gln Ser Pro Ala
375 380 385

CTG GAA TTA GAT AAA TTC CTT GAA GAT GTC AGG AAT GGG ATC TAT CCT 1374
Leu Glu Leu Asp Lys Phe Leu Glu Asp Val Arg Asn Gly Ile Tyr Pro
390 395 400

CTG ACA GCC TTT GGG CTG CCT CGG CCC CAG CAG CCA CAG CAG GAG GAG 1422
Leu Thr Ala Phe Gly Leu Pro Arg Pro Gln Gln Pro Gln Gln Glu Glu
405 410 415

GTG ACA TCA CCT GTC GTG CCC CCC TCT GTC AAG ACT CCG ACA CCT GAA 1470
Val Thr Ser Pro Val Val Pro Pro Ser Val Lys Thr Pro Thr Pro Glu
420 425 430 435

CCA GCT GAG GTG GAG ACT CGC AAG GTG GTG CTG ATG CAG TGC AAC ATT 1518
Pro Ala Glu Val Glu Thr Arg Lys Val Val Leu Met Gln Cys Asn Ile
440 445 450

GAG TCG GTG GAG GAG GGA GTC AAA CAC CAC CTG ACA CTT CTG CTG AAG 1566
Glu Ser Val Glu Glu Gly Val Lys His His Leu Thr Leu Leu Leu Lys
455 460 465

TTG GAG GAC AAA CTG AAC CGG CAC CTG AGC TGT GAC CTG ATG CCA AAT 1614
Leu Glu Asp Lys Leu Asn Arg His Leu Ser Cys Asp Leu Met Pro Asn
470 475 480

```
GAG AAT ATC CCC GAG TTG GCG GCT GAG CTG GTG CAG CTG GGC TTC ATT      1662
Glu Asn Ile Pro Glu Leu Ala Ala Glu Leu Val Gln Leu Gly Phe Ile
    485                 490                 495

AGT GAG GCT GAC CAG AGC CGG TTG ACT TCT CTG CTA GAA GAG ACC TTG      1710
Ser Glu Ala Asp Gln Ser Arg Leu Thr Ser Leu Leu Glu Glu Thr Leu
500                 505                 510                 515

AAC AAG TTC AAT TTT GCC AGG AAC AGT ACC CTC AAC TCA GCC GCT GTC      1758
Asn Lys Phe Asn Phe Ala Arg Asn Ser Thr Leu Asn Ser Ala Ala Val
                520                 525                 530

ACC GTC TCC TCT TAGAGCTCAC TCGGGCCAGG CCCTGATCTG CGCTGTGGCT          1810
Thr Val Ser Ser
            535

GTCCCTGGAC GTGCTGCAGC CCTCCTGTCC CTTCCCCCCA GTCAGTATTA CCCTGTGAAG    1870

CCCCTTCCCT CCTTTATTAT TCAGGAGGGC TGGGGGGGCT CCCTGGTTCT GAGCATCATC    1930

CTTTCCCCTC CCCTCTCTTC CTCCCCTCTG CACTTTGTTT ACTTGTTTTG CACAGACGTG    1990

GGCCTGGGCC TTCTCAGCAG CCGCCTTCTA GTTGGGGGCT AGTCGCTGAT CTGCCGGCTC    2050

CCGCCCAGCC TGTGTGGAAA GGAGGCCCAC GGGCACTAGG GGAGCCGAAT TCTACAATCC    2110

CGCTGGGGCG GCCGGGGCGG GAGAGAAAGG TGGTGCTGCA GTGGTGGCCC TGGGGGGCCA    2170

TTCGATTCGC CTCAGTTGCT GCTGTAATAA AAGTCTACTT TTTGCTAAAA AAAAAAAAAA    2230

AAAAAAAAAA A                                                         2241
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 535 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Ser Glu Gly Glu Ser Gln Thr Val Leu Ser Ser Gly Ser Asp Pro
```

1 5 10 15

Lys Val Glu Ser Ser Ser Ser Ala Pro Gly Leu Thr Ser Val Ser Pro
20 25 30

Pro Val Thr Ser Thr Thr Ser Ala Ala Ser Pro Glu Glu Glu Glu Glu
35 40 45

Ser Glu Asp Glu Ser Glu Ile Leu Glu Glu Ser Pro Cys Gly Arg Trp
50 55 60

Gln Lys Arg Arg Glu Glu Val Asn Gln Arg Asn Val Pro Gly Ile Asp
65 70 75 80

Ser Ala Tyr Leu Ala Met Asp Thr Glu Glu Gly Val Glu Val Val Trp
85 90 95

Asn Glu Val Gln Phe Ser Glu Arg Lys Asn Tyr Lys Leu Gln Glu Glu
100 105 110

Lys Val Cys Ala Val Phe Asp Asn Leu Ile Gln Leu Glu His Leu Asn
115 120 125

Ile Val Lys Phe His Lys Tyr Trp Ala Asp Ile Lys Glu Asn Lys Ala
130 135 140

Arg Val Ile Phe Ile Thr Gly Tyr Met Ser Ser Gly Ser Leu Lys Gln
145 150 155 160

Phe Leu Lys Lys Thr Gln Lys Asn His Gln Thr Met Asn Glu Lys Ala
165 170 175

Trp Lys Arg Trp Cys Thr Gln Ile Leu Ser Ala Leu Ser Tyr Leu His
180 185 190

Ser Cys Asp Pro Pro Ile Ile His Gly Asn Leu Thr Cys Asp Thr Ile
195 200 205

Phe Ile Gln His Asn Gly Leu Ile Lys Ile Gly Ser Val Ala Pro Asp
210 215 220

Thr Ile Asn Asn His Val Lys Thr Cys Arg Glu Glu Gln Lys Asn Leu
225 230 235 240

His Phe Phe Ala Pro Glu Tyr Gly Glu Val Thr Asn Val Thr Thr Ala

245 250 255

Val Asp Ile Tyr Ser Phe Gly Met Cys Ala Leu Gly Met Ala Val Leu
260 265 270

Glu Ile Gln Gly Asn Gly Glu Ser Ser Tyr Val Pro Gln Glu Ala Ile
275 280 285

Ser Ser Ala Ile Gln Leu Leu Glu Asp Pro Leu Gln Arg Glu Phe Ile
290 295 300

Gln Lys Cys Leu Gln Ser Glu Pro Ala Arg Arg Pro Thr Ala Arg Glu
305 310 315 320

Leu Leu Phe His Pro Ala Leu Phe Glu Val Pro Ser Leu Lys Leu Leu
325 330 335

Ala Ala His Cys Ile Val Gly His Gln His Met Ile Pro Glu Asn Ala
340 345 350

Leu Glu Glu Ile Thr Lys Asn Met Asp Thr Ser Ala Val Leu Ala Glu
355 360 365

Ile Pro Ala Gly Pro Gly Arg Glu Pro Val Gln Thr Leu Tyr Ser Gln
370 375 380

Ser Pro Ala Leu Glu Leu Asp Lys Phe Leu Glu Asp Val Arg Asn Gly
385 390 395 400

Ile Tyr Pro Leu Thr Ala Phe Gly Leu Pro Arg Pro Gln Gln Pro Gln
405 410 415

Gln Glu Glu Val Thr Ser Pro Val Val Pro Pro Ser Val Lys Thr Pro
420 425 430

Thr Pro Glu Pro Ala Glu Val Glu Thr Arg Lys Val Val Leu Met Gln
435 440 445

Cys Asn Ile Glu Ser Val Glu Glu Gly Val Lys His His Leu Thr Leu
450 455 460

Leu Leu Lys Leu Glu Asp Lys Leu Asn Arg His Leu Ser Cys Asp Leu
465 470 475 480

Met Pro Asn Glu Asn Ile Pro Glu Leu Ala Ala Glu Leu Val Gln Leu

485 490 495

Gly Phe Ile Ser Glu Ala Asp Gln Ser Arg Leu Thr Ser Leu Leu Glu
500 505 510

Glu Thr Leu Asn Lys Phe Asn Phe Ala Arg Asn Ser Thr Leu Asn Ser
515 520 525

Ala Ala Val Thr Val Ser Ser
530 535

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCACAGTCGA CCAAGCCGGA GTCGCAGAG 39

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCACAAAGCT TGCCAGGAGG GGTCTGGCTG 30

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCACAACCAT GGCCAAGCCG GAGTCGCAGG AG 32

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCACAAGATC TCCAGGAGGG GTCTGGCTG 29

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Lys Pro Glu Ser Gln Glu Ala Ala Pro Leu Ser Gly Pro Cys

5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Glu Asp Ala Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser Leu Arg Leu Trp Asp
                1                   10                  15
Cys
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Gly Tyr Ile Lys Glu Asp Glu Ala Leu Pro Ser Pro His Thr Leu Gln Cys
                5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCACAGGTAC CGAGGCCATG GGCGCGCGC 29

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCACATCTAG ATCAGTGGTG GTGGTGGTGG TGGACCGGCC CCAGGAGTGG 50

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCACAGCGGC CGCGAGGCCA TGGGCGCGCG C 31

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCACAGCGGC CGCTCAGTGG TGGTGGTGGT GGTGCCAGGA GGGGTCTGGC TG 52

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTGACTTCCA TGCCATCCTT 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCTCACGACT CCAATCTGAT 20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Arg Ile Val Gly Gly
5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 959 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..856

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
C GAC CTA TTG TCA GGG CCC TGC GGT CAC AGG ACC ATC CCT TCC CGT      46
  Asp Leu Leu Ser Gly Pro Cys Gly His Arg Thr Ile Pro Ser Arg
    1               5                  10                  15

ATA GTG GGT GGC GAT GAT GCT GAG CTT GGC CGC TGG CCG TGG CAA GGG    94
Ile Val Gly Gly Asp Asp Ala Glu Leu Gly Arg Trp Pro Trp Gln Gly
                20                  25                  30

AGC CTG CGT GTA TGG GGC AAC CAC TTA TGT GGC GCA ACC TTG CTC AAC   142
Ser Leu Arg Val Trp Gly Asn His Leu Cys Gly Ala Thr Leu Leu Asn
            35                  40                  45

CGC CGC TGG GTG CTT ACA GCT GCC CAC TGC TTC CAA AAG GAT AAC GAT   190
Arg Arg Trp Val Leu Thr Ala Ala His Cys Phe Gln Lys Asp Asn Asp
        50                  55                  60

CCT TTT GAC TGG ACA GTC CAG TTT GGT GAG CTG ACT TCC AGG CCA TCT   238
Pro Phe Asp Trp Thr Val Gln Phe Gly Glu Leu Thr Ser Arg Pro Ser
    65                  70                  75

CTC TGG AAC CTA CAG GCC TAT TCC AAC CGT TAC CAA ATA GAA GAT ATT   286
Leu Trp Asn Leu Gln Ala Tyr Ser Asn Arg Tyr Gln Ile Glu Asp Ile
80                  85                  90                  95

TTC CTG AGC CCC AAG TAC TCG GAG CAG TAT CCC AAT GAC ATA GCC CTG   334
Phe Leu Ser Pro Lys Tyr Ser Glu Gln Tyr Pro Asn Asp Ile Ala Leu
                100                 105                 110

CTG AAG CTG TCA TCT CCA GTC ACC TAC AAT AAC TTC ATC CAG CCC ATC   382
Leu Lys Leu Ser Ser Pro Val Thr Tyr Asn Asn Phe Ile Gln Pro Ile
            115                 120                 125
```

TGC CTC CTG AAC TCC ACG TAC AAG TTT GAG AAC CGA ACT GAC TGC TGG 430
Cys Leu Leu Asn Ser Thr Tyr Lys Phe Glu Asn Arg Thr Asp Cys Trp
130 135 140

GTG ACC GGC TGG GGG GCT ATT GGA GAA GAT GAG AGT CTG CCA TCT CCC 478
Val Thr Gly Trp Gly Ala Ile Gly Glu Asp Glu Ser Leu Pro Ser Pro
145 150 155

AAC ACT CTC CAG GAA GTG CAG GTA GCT ATT ATC AAC AAC AGC ATG TGT 526
Asn Thr Leu Gln Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met Cys
160 165 170 175

AAC CAT ATG TAC AAA AAG CCA GAC TTC CGC ACG AAC ATC TGG GGA GAC 574
Asn His Met Tyr Lys Lys Pro Asp Phe Arg Thr Asn Ile Trp Gly Asp
180 185 190

ATG GTT TGC GCT GGC ACT CCT GAA GGT GGC AAG GAT GCC TGC TTT GGT 622
Met Val Cys Ala Gly Thr Pro Glu Gly Gly Lys Asp Ala Cys Phe Gly
195 200 205

GAC TCG GGA GGA CCC TTG GCC TGC GAC CAG GAT ACG GTG TGG TAT CAG 670
Asp Ser Gly Gly Pro Leu Ala Cys Asp Gln Asp Thr Val Trp Tyr Gln
210 215 220

GTT GGA GTT GTG AGC TGG GGA ATA GGC TGT GGT CGC CCC AAT CGC CCT 718
Val Gly Val Val Ser Trp Gly Ile Gly Cys Gly Arg Pro Asn Arg Pro
225 230 235

GGA GTC TAT ACC AAC ATC AGT CAT CAC TAC AAC TGG ATC CAG TCA ACC 766
Gly Val Tyr Thr Asn Ile Ser His His Tyr Asn Trp Ile Gln Ser Thr
240 245 250 255

ATG ATC CGC AAT GGG CTG CTC AGG CCT GAC CCA GTC CCC TTG CTA CTG 814
Met Ile Arg Asn Gly Leu Leu Arg Pro Asp Pro Val Pro Leu Leu Leu
260 265 270

TTT CTT ACT CTG GCC TGG GCT TCC TCT TTG CTG AGG CCT GCC 856
Phe Leu Thr Leu Ala Trp Ala Ser Ser Leu Leu Arg Pro Ala
275 280 285

TGAGCCCACA CGTGTACGTC ACACCTGTGA GGTCAGGGTG TGTCTCTTTT GTATCTTGCT 916

TGCTAATAAA CCTGTTAATA TTTAAAAAAA AAAAAAAAAA AAA 959

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Asp Leu Leu Ser Gly Pro Cys Gly His Arg Thr Ile Pro Ser Arg Ile
 1               5                  10                  15

Val Gly Gly Asp Asp Ala Glu Leu Gly Arg Trp Pro Trp Gln Gly Ser
            20                  25                  30

Leu Arg Val Trp Gly Asn His Leu Cys Gly Ala Thr Leu Leu Asn Arg
        35                  40                  45

Arg Trp Val Leu Thr Ala Ala His Cys Phe Gln Lys Asp Asn Asp Pro
    50                  55                  60

Phe Asp Trp Thr Val Gln Phe Gly Glu Leu Thr Ser Arg Pro Ser Leu
65                  70                  75                  80

Trp Asn Leu Gln Ala Tyr Ser Asn Arg Tyr Gln Ile Glu Asp Ile Phe
                85                  90                  95

Leu Ser Pro Lys Tyr Ser Glu Gln Tyr Pro Asn Asp Ile Ala Leu Leu
            100                 105                 110

Lys Leu Ser Ser Pro Val Thr Tyr Asn Asn Phe Ile Gln Pro Ile Cys
        115                 120                 125

Leu Leu Asn Ser Thr Tyr Lys Phe Glu Asn Arg Thr Asp Cys Trp Val
    130                 135                 140

Thr Gly Trp Gly Ala Ile Gly Glu Asp Glu Ser Leu Pro Ser Pro Asn
145                 150                 155                 160

Thr Leu Gln Glu Val Gln Val Ala Ile Ile Asn Asn Ser Met Cys Asn
                165                 170                 175

His Met Tyr Lys Lys Pro Asp Phe Arg Thr Asn Ile Trp Gly Asp Met
```

180 185 190

Val Cys Ala Gly Thr Pro Glu Gly Gly Lys Asp Ala Cys Phe Gly Asp
195 200 205

Ser Gly Gly Pro Leu Ala Cys Asp Gln Asp Thr Val Trp Tyr Gln Val
210 215 220

Gly Val Val Ser Trp Gly Ile Gly Cys Gly Arg Pro Asn Arg Pro Gly
225 230 235 240

Val Tyr Thr Asn Ile Ser His His Tyr Asn Trp Ile Gln Ser Thr Met
245 250 255

Ile Arg Asn Gly Leu Leu Arg Pro Asp Pro Val Pro Leu Leu Leu Phe
260 265 270

Leu Thr Leu Ala Trp Ala Ser Ser Leu Leu Arg Pro Ala
275 280 285

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3866 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AGTGAGTCTC CTGCCTCAGC CTCCCAAGTA GCTGGGACTT CAGGTGTGTG CCACCATCCT 60

CAGCTAATTT TTTTTTTTTT TTTTTTTTTG AGAAGGAGTC TTGCTCTGTC GCCCAGGCTG 120

GAGTGCAGTG GCGCGATCTT CCAGGCCCCA CCGGGCCCTC AGGAAGGCCT TGCCTACCTG 180

CTTTAAGGGG ACTCCTGGCT CAGGGCCAGG CCCCTGGTGC TGGAGGAGGT GGTGGGTGGA 240

GGGCAGGGGG CACCAAGCGG GCAGCCAGGA CCCCCGGGCT GCAGACAAGA AAAGGACTGT 300

GGGGTCCACC GGGTCTGGGC CACATCAAGG AATGTGGTTG AAGACCCGCC CTTAGGAGCT 360

GAAAGCCAGG GCGCTACCAG GCCTGAGAGG CCCCAAACAG CCCTTGGGCC TGGTTTGGGA 420

GGATTAAGCT GGAGCTCCCA ACCCGCCCTG CCCCCAGGGG GCGACCCCGG GCCCGGCGCG 480

AGAGGAGGCA GAGGGGGCGT CAGGCCGCGG GAGAGGAGGC CATGGGCGCG CGCGGGGCGC 540

TGCTGCTGGC GCTGCTGCTG GCTCGGGCTG GACTCAGGAA GCCGGGTGAG CTCGGGGCGC 600

TGCTGGCGGG ATGGGGAGGC GGGGGAGCGG TGGGGAGGAC GGGAGGTGGA GGCCGCGGGG 660

AGTCACTTCT TGTCTCCCGC AGAGTCGCAG GAGGCGGCGC CGTTATCAGG TAGGGCGCCC 720

AGGACGCGCG ATTCCTGCCA GGGCCGTTGG GCCGAGGTGG ACGGGGGGCG GTGAGGGGGT 780

AGAGGGGGGC CTTTACTGCT CTCTCGCCCC CGCCCCCGGG ATCGAGAACT CTGTTGGCGT 840

GGAAAGTAAC TAACGGACGC TGGAGGGGGA TGGGCGGGCC CTGCAGAGCA CGTGGGAGGA 900

TCTCCAGTGT CACCTACTTC CTGCTGCACA CACGCGAGGG GACCCTGGGT GGGCAAAAAC 960

GTGCTTTCCC GGACGGGGTT GAAGGGGAGA AAGGGAGAGG TCGGGCTTGG GGGGCTGCCT 1020

CCCGCGGCTC AGCAGTTCCT CTGACCATCC GAGGACCATG CGGCCGACGG GTCATCACGT 1080

CGCGCATCGT GGGTGGAGAG GACGCCGAAC TCGGGCGTTG GCCGTGGCAG GGGAGCCTGC 1140

GCCTGTGGGA TTCCCACGTA TGCCGAGTGA GCCTGCTCAG CCACCGCTGG GCACTCACGG 1200

CGGCGCACTG CTTTGAAACG TGAGTGGGGG TGCGAACGGA GGGGTGCGGG GACGGGCAGG 1260

AACAGGGCTG GAGGGAGTGC CACCGAACTT TACCTCTGGT CTGATGCCAG ACTTGGGCGT 1320

GAAAGTTGTG CGTGGATGCG GCCTGGTGTT CTCCTGAGCC CCAGGCTGTG CTGCAGCCGG 1380

TTACACCCAC TCCAGTTCCC TTTGGGTCTC CTGGAGGGAA CCCTGTTCAG GTTATTCCAG 1440

AATGTTCTTC CAGAACATTT CCACACACTT TTGGGTATTC TCTCCCTTTT TCTTTCAACC 1500

CAAAGTTCAC CACTGACCAT CCCACCCTCA TCCCCCCTCC TGGTGGACGG TGCGGTACAG 1560

TGTGGGGCAC TGAGCCAAGG CCAGCACCCC CGGGCCGCTG TGTGGACTCC ATCCTGCCAA 1620

TCCCACATTG GCGTGGTGCA TCTCCCCATT CCTCCTTGGG CTGCATGGGG GTGCCCCTGG 1680

AGGCCTTGGC TCAATGCAAG GCTCCTTGGG ACAGCTCTGG GAGGTGACAA GACCCCACCC 1740

TTCTGCTGCA GGAGCAGGTC CTAGGACTTT GGTTGTGGTC TGTCTGGGCT CCTTCATTTC 1800

TGCAGGGGAC CCTGGGTGTT AGCAAGTAGC AGCAACACCA CAGTTTCCCC TCCTGCACTG 1860

GACCCCAGTT GTGCTCAGGT AGCCAGCCCT CCATCCAGGG CCCCTGACTG CTCTCTTCTC 1920

TTCTGCCAGC TATAGTGACC TTAGTGATCC CTCCGGGTGG ATGGTCCAGT TTGGCCAGCT 1980

GACTTCCATG CCATCCTTCT GGAGCCTGCA GGCCTACTAC ACCCGTTACT TCGTATCGAA 2040

TATCTATCTG AGCCCTCGCT ACCTGGGGAA TTCACCCTAT GACATTGCCT TGGTGAAGCT 2100

GTCTGCACCT GTCACCTACA CTAAACACAT CCAGCCCATC TGTCTCCAGG CCTCCACATT 2160

TGAGTTTGAG AACCGGACAG ACTGCTGGGT GACTGGCTGG GGGTACATCA AAGAGGATGA 2220

GGGTGAGGCT GGGGACAGGC GGGTCAGGGA GGAACTGTCT TTGTTCACCT GTTCCCCTGC 2280

ATAGGCACAA TAGCCCCCTG CTTGGTCTGG GGGTGCAGGC TATGCCCCTC TTGCTTGCAG 2340

TCTCTCCTCA CCTGCCAGGG CAGGGACCAA ACACCCAGTT CTCTCCCTTC CAGGGGCTGT 2400

GGGGGCCAGA AGGAGAGTGT GAGAGGGAGG CCAGTTTGGC GCAAGCCTGT GGGTGGTGCG 2460

GTGGTGGAGG GGTTCTGGAG GGCTTGGCGA CATAAACCTC ATACTTGGAT TTATTCCTGC 2520

ATCTTTCCAC CTCCCCCAGT GCTCACCAAT GCCCCAGGCA TCACCAGCTT GCCCCTTCCC 2580

CCAAGGTCTG GCTTTGGATG CTTATGTGAA CACCGTTTTA AGTTGCCTTG GCCCCTTCCT 2640

CGGTTCCTTT TTGGCTGAGG AATCTCTCCA TGGCTGCAGG CAGGGCCATT GTTGCCATTC 2700

TACAGATAGG GAAAGTGCGG CTGGGGGAGC TCTGACAGCT GTCCCTCCCC GGGGCCTTCT 2760

GTGATGCTGC TGAGGGCCTC TGTTGTGCTG GGGTCTGGGT TGGAGCTGGG GGTAATGGAG 2820

ATGAACCTGC CAGGCACAGT GGGTGCCCCA GGGCCCCCAC CCCCGCAGCC TATGCCATCC 2880

CTCCATAGAG GGGCCTCAGG TTGCTGTCTC TCTCCTTCCC ACTATCGTCC GCACAGCACT 2940

GCCATCTCCC CACACCCTCC AGGAAGTTCA GGTCGCCATC ATAAACAACT CTATGTGCAA 3000

CCACCTCTTC CTCAAGTACA GTTTCCGCAA GGACATCTTT GGAGACATGG TTTGTGCTGG 3060

CAATGCCCAA GGCGGGAAGG ATGCCTGCTT CGTGAGTGTC CTTGCCACCA CTCCCAGCCC 3120

AGGAAAGCAT CCTGTGTCCC TGTGCCTTAT TTGACCCTCA TGCCAACCCC GGGAGGTGGA 3180

GACTGTTGCC CCACTCTGCA GATGCAGAAA CGGAGGCTTG GCTGCTGCCA GGGGGAGGAG 3240

GAGGATGTGC ACCCAGTCTA CCCAGCCCCA TAGCCCTTCC CACTCTCAGC CCCTCCCCTG 3300

CCCCACTCAC TCTGCCCCAG GCTGACCTCA GCCCCGCTGC TCCCCAGGGT GACTCAGGTG 3360

GACCCTTGGC CTGTAACAAG AATGGACTGT GGTATCAGAT TGGAGTCGTG AGCTGGGGAG 3420

TGGGCTGTGG TCGGCCCAAT CGGCCCGGTG TCTACACCAA TATCAGCCAC CACTTTGAGT 3480

GGATCCAGAA GCTGATGGCC CAGAGTGGCA TGTCCCAGCC AGACCCCTCC TGGCCGCTAC 3540

TCTTTTTCCC TCTTCTCTGG GCTCTCCCAC TCCTGGGGCC GGTCTGAGCC TACCTGAGCC 3600

CATGCAGCCT GGGGCCACTG CCAAGTCAGG CCCTGGTTCT CTTCTGTCTT GTTTGGTAAT 3660

AAACACATTC CAGTTGATGC CTTGCAGGGC ATTCTTCAAA AGCAGTGGCT TCATGGACAG 3720

CTCATTCTCT CTTGTGCAGA CAGCCTGTCT GTGCCCCTGG CTCACACCCA CATCTGTTCT 3780

GCACCATAGA ACCATCTGGT TATTTCGATC AGAAAGAGAA TTGTGTGTTG CCCAGGCTGG 3840

TCTTGAACGC CTAGGGTGTC TCGATC 3866

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1165 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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CTGAACCGGG TTGTGGGCGG CGAGGACAGC ACTGACAGCG AGTGGCCCTG GATCGTGAGC     60
ATCCAGAAGA ATGGGACCCA CCACTGCGCA GGTTCTCTGC TCACCAGCCG CTGGGTGATC    120
ACTGCTGCCC ACTGTTTCAA GGACAACCTG AACAAACCAT ACCTGTTCTC TGTGCTGCTG    180
GGGGCCTGGC AGCTGGGGAA CCCTGGCTCT CGGTCCCAGA AGGTGGGTGT TGCCTGGGTG    240
GAGCCCCACC CTGTGTATTC CTGGAAGGAA GGTGCCTGTG CAGACATTGC CCTGGTGCGT    300
CTCGAGCGCT CCATACAGTT CTCAGAGCGG GTCCTGCCCA TCTGCCTACC TGATGCCTCT    360
ATCCACCTCC CTCCAAACAC CCACTGCTGG ATCTCAGGCT GGGGGAGCAT CCAAGATGGA    420
GTTCCCTTGC CCCACCCTCA GACCCTGCAG AAGCTGAAGG TTCCTATCAT CGACTCGGAA    480
GTCTGCAGCC ATCTGTACTG GCGGGGAGCA GGACAGGGAC CCATCACTGA GGACATGCTG    540
TGTGCCGGCT ACTTGGAGGG GGAGCGGGAT GCTTGTCTGG GCGACTCCGG GGGCCCCCTC    600
ATGTGCCAGG TGGACGGCGC CTGGCTGCTG GCCGGCATCA TCAGCTGGGG CGAGGGCTGT    660
GCCGAGCGCA ACAGGCCCGG GGTCTACATC AGCCTCTCTG CGCACCGCTC CTGGGTGGAG    720
AAGATCGTGC AAGGGGTGCA GCTCCGCGGG CGCGCTCAGG GGGGTGGGGC CCTCAGGGCA    780
CCGAGCCAGG GCTCTGGGGC CGCCGCGCGC TCCTAGGGCG CAGCGGGACG CGGGGCTCGG    840
ATCTGAAAGG CGGCCAGATC CACATCTGGA TCTGGATCTC CGGCGGCCTC GGGCGGTTTC    900
CCCCGCCGTA AATAGGCTCA TCTACCTCTA CCTCTGGGGG CCCGGACGGC TGCTGCGGAA    960
AGGAAACCCC CTCCCCGACC CGCCCGACGG CCTCAGGCCC CGCCTCCAAG GCATCAGGCC   1020
CCGCCCAACG GCCTCATGTC CCCGCCCCCA CGACTTCCGG CCCCGCCCCG GGCCCCAGCG   1080
CTTTTGTGTA TATAAATGTT AATGATTTTT ATAGGTATTT GTAACCCTGC CCACATATCT   1140
TATTTATTCC TCCAATTTCA ATAAA                                         1165
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(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 933 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

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AATGCGGCCA CTCCAAGGAG GCCGGGAGGA TTGTGGGAGG CCAAGACACC CAGGAAGGAC     60
GCTGGCCGTG GCAGGTTGGC CTGTGGTTGA CCTCAGTGGG GCATGTATGT GGGGGCTCCC    120
TCATCCACCC ACGCTGGGTG CTCACAGCCG CCCACTGCTT CCTGAGGTCT GAGGATCCCG    180
GGCTCTACCA TGTTAAAGTC GGAGGGCTGA CACCCTCACT TTCAGAGCCC CACTCGGCCT    240
TGGTGGCTGT GAGGAGGCTC CTGGTCCACT CCTCATACCA TGGGACCACC ACCAGCGGGG    300
ACATTGCCCT GATGGAGCTG GACTCCCCCT TGCAGGCCTC CCAGTTCAGC CCCATCTGCC    360
TCCCAGGACC CCAGACCCCC CTCGCCATTG GGACCGTGTG CTGGGTAAAC GGGCTGGGGG    420
TCCACTCAGG AGAGGCCCTG GCGAGTGTCC TTCAGGAGGT GGCTGTGCCC CTCCTGGACT    480
CGAACATGTG TGAGCTGATG TACCACCTAG GAGAGCCCAG CCTGGCTGGC CAGCGCCTCA    540
TCCAGGACGA CATGCTCTGT GCTGGCTCTG TCCAGGGCAA GAAAGACTCC TGCCAGGGTG    600
ACTCCGGGGG GCCGCTGGTC TGCCCCATCA ATGATACGTG GATCCAGGCC GGCATTGTGA    660
GCTGGGGATT CCGCTGTGCC CGGCCTTTCC GGCCTGGTGT CTACACCCAG GTGCTAAGCT    720
ACACAGACTG GATTCAGAGA ACCCTGGCTG AATCTCACTC AGGCATGTCT GGGGCCCGCC    780
CAGGTGCCCC AGGATCCCAC TCAGGCACCT CCAGATCCCA CCCAGTGCTG CTGCTTGAGC    840
TGTTGACCGT ATGCTTGCTT GGGTCCCTGT GAACCATGAG CCATGGAGTC CGGGATCCCC    900
TTTCTGGTAG GATTGATGGA ATCTAATAAT AAA                                 933
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 980 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
CCTGTGGTCG CCCCAGGATG CTGAACCGAA TGGTGGGCGG GCAGGACACG CAGGAGGGCG    60
AGTGGCCCTG GCAAGTCAGC ATCCAGCGCA ACGGAAGCCA CTTCTGCGGG GGCAGCCTCA   120
TCGCGGAGCA GTGGGTCCTG ACGGCTGCGC ACTGCTTCCG CAACACCTCT GAGACGTCCC   180
TGTACCAGGT CCTGCTGGGG GCAAGGCAGC TAGTGCAGCC GGGACCACAC GCTATGTATG   240
CCCGGGTGAG GCAGGTGGAG AGCAACCCCC TGTACCAGGG CACGGCCTCC AGCGCTGACG   300
TGGCCCTGGT GGAGCTGGAG GCACCAGTGC CCTTCACCAA TTACATCCTC CCCGTGTGCC   360
TGCCTGACCC CTCGGTGATC TTTGAGACGG GCATGAACTG CTGGGTCACT GGCTGGGGCA   420
GCCCCAGTGA GGAAGACCTC CTGCCCGAAC CGCGGATCCT GCAGAAACTC GCTGTGCCCA   480
TCATCGACAC ACCCAAGTGC AACCTGCTCT ACAGCAAAGA CACCGAGTTT GGCTACCAAC   540
CCAAAACCAT CAAGAATGAC ATGCTGTGCG CCGGCTTCGA GGAGGGCAAG AAGGATGCCT   600
GCAAGGGCGA CTCGGGCGGC CCCCTGGTGT GCCTCGTGGG TCAGTCGTGG CTGCAGGCGG   660
GGGTGATCAG CTGGGGTGAG GGCTGTGCCC GCCAGAACCG CCCAGGTGTC TACATCCGTG   720
TCACCGCCCA CCACAACTGG ATCCATCGGA TCATCCCCAA ACTGCAGTTC CAGCCAGCGA   780
GGTTGGGCGG CCAGAAGTGA GACCCCCGGG GCCAGGAGCC CCTTGAGCAG AGCTCTGCAC   840
CCAGCCTGCC CGCCCACACC ATCCTGCTGG TCCTCCCAGC GCTGCTGTTG CACCTGTGAG   900
CCCCACCAGA CTCATTTGTA AATAGCGCTC CTTCCTCCCC TCTCAAATAC CCTTATTTTA   960
TTTATGTTTC TCCCAATAAA                                               980
```

CLAIMS:

1. An isolated proteinaceous molecule involved in or associated with regulation of cell activity and/or viability comprising a sequence of amino acids encoded by a nucleotide sequence, at least a portion of which, is capable of being amplified by polymerase chain reaction (PCR) using the following primers:

5′ ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3′ [SEQ ID NO:1]; and

5′ACAGAATTCAXIGGICCICCIC/GT/AXTCICC3′ [SEQ ID NO:2];

or a complementary form of said primers.

2. An isolated proteinaceous molecule according to claim 1 wherein said molecule is a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:4 or an amino acid sequence having at least 50% similarity thereto.

3. An isolated proteinaceous molecule according to claim 1 wherein said molecule is a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least 50% similarity thereto.

4. An isolated proteinase molecule according to claim 1 wherein said molecule is a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity thereto.

5. An isolated proteinaceous molecule according to claim 1 wherein said molecule is a serine proteinase comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions at 42°C.

6. An isolated proteinaceous molecule according to claim 1 wherein said molecule is a serine proteinase comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions at 42°C.

7. An isolated proteinaceous molecule according to claim 1 wherein said molecule is a serine proteinase comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:7 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:7 under low stringency conditions at 42°C.

8. An isolated proteinaceous molecule according to claim 1 wherein said molecule is a kinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:10 or having 50% amino acid similarity thereto.

9. An isolated proteinaceous molecule according to claim 1 wherein said molecule is a kinase comprising an amino acid sequence encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:9 under low stringency conditions at 42°C.

10. An isolated nucleic acid molecule encoding a polypeptide wherein at least a portion of said nucleic acid molecule is capable of being amplified by polymerase chain reaction (PCR) using the following primers:

5′ ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3′ [SEQ ID NO:1]; and

5′ACAGAATTCAXIGGICCICCIC/GT/AXTCICC3′ [SEQ ID NO:2];

or a complementary form of said primers.

11. An isolated nucleic acid molecule according to claim 10 wherein said polypeptide is a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:4 or an amino acid sequence having at least 50% similarity thereto.

12. An isolated nucleic acid molecule according to claim 10 wherein said polypeptide is a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least 50% similarity thereto.

13. An isolated nucleic acid molecule according to claim 10 wherein said polypeptide is a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity thereto.

14. An isolated nucleic acid molecule according to claim 10 comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions at 42°C.

15. An isolated nucleic acid molecule according to claim 10 comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions at 42°C.

16. An isolated nucleic acid molecule according to claim 10 comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:7 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:7 under low stringency conditions at 42°C.

17. An isolated nucleic acid molecule according to claim 10 wherein said polypeptide is a kinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:10 or having 50% amino acid similarity thereto.

18. An isolated nucleic acid molecule according to claim 17 comprising a sequence of nucleotides encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:9 under low stringency conditions at 42°C.

19. An isolated serine proteinase encoded by a gene proximal to a cluster of genes of a mammalian chromosome.

20. An isolated serine proteinase according to claim 19 wherein the mammalian chromosome is human chromosome 16p13.3 or its equivalent in a non-human species.

21. An isolated serine proteinase according to claim 20 wherein the gene cluster includes at least two genes having the nucleotide sequence as set forth in SEQ ID NO:3 or 5 or 28 or 29 or 30 or a nucleotide sequence having at least 50% similarity to any one of SEQ ID NO:3 or 5 or 28 or 29 or 30 or a nucleotide sequence capable of hybridizing to any one of the sequences under low stringency conditions at 42°C.

22. An isolated serine proteinase according to claim 20 wherein said serine proteinase is a short form of HELA2 having an amino acid sequence substantially as set forth in SEQ ID NO:4 or an amino acid sequence having at least 50% similarity thereto.

23. An isolated serine proteinase according to claim 20 wherein said serine proteinase is a long form of HELA2 having an amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least 50% similarity thereto.

24. An isolated serine proteinase according to claim 22 encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 50% similarity thereto or a sequence capable of hybridizing to SEQ I NO:3 under low stringency conditions at 42°C.

25. An isolated serine proteinase according to claim 23 encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity thereto or a sequence capable of hybridizing to SEQ ID NO:5 under low stringency conditions at 42°C.

26. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a serine proteinase and corresponding to a gene proximal to a cluster of genes encoding serine proteinases.

27. An isolated nucleic acid molecule according to claim 26 wherein the gene cluster includes at least two genes having the nucleotide sequence as set forth in SEQ ID NO:3 or 5 or 28 or 29 or 30 or a nucleotide sequence having at least 50% similarity to any one of SEQ ID NO:3 or 5 or 28 or 29 or 30 or a nucleotide sequence capable of hybridizing to any one of the sequences under low stringency conditions at 42°C.

28. An isolated nucleic acid molecule according to claim 25 comprising a nucleotide sequence substantially as set forth in SEQ ID NO:3 or SEQ ID NO:5 or a nucleotide sequence having at least about 50% similarity to either of SEQ ID NO:3 or SEQ ID NO:5 or a nucleotide sequence capable of hybridizing to SEQ ID NO:3 or SEQ ID NO:5 under low stringency conditions at 42°C.

29. An isolated kinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:10 or an amino acid sequence having at least about 50% similarity thereto.

30. An isolated kinase according to claim 29 encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity thereto or capable of hybridizing to SEQ ID NO:9 under low stringency conditions at 42°C.

31. A method of regulating cell activity and/or viability said method comprising contacting said cell with an activity and/or viability effective amount of a serine proteinase and/or kinase.

32. A method according to claim 31 wherein the serine proteinase comprises a sequence of amino acids encoded by a nucleotide sequence, at least a portion of which, is capable of being amplified by polymerase chain reaction (PCR) using the following primers:

5′ ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3′ [SEQ ID NO:1]; and

5′ACAGAATTCAXIGGICCICCIC/GT/AXTCICC3′ [SEQ ID NO:2];

or a complementary form of said primers.

33. A method according to claim 31 wherein the serine proteinase comprises an amino acid sequence substantially as set forth in SEQ ID NO:4 or an amino acid sequence having at least 50% similarity thereto.

34. A method according to claim 31 wherein the serine proteinase comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least 50% similarity thereto.

35. A method according to claim 31 wherein the serine proteinase comprises an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity thereto.

36. A method according to claim 31 wherein the serine proteinase comprises a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions at 42°C.

37. A method according to claim 31 wherein the serine proteinase comprises a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions at 42°C.

38. A method according to claim 31 wherein the serine proteinase comprises a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:7 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:7 under low stringency conditions at 42°C.

39. A method according to claim 31 wherein the kinase comprises an amino acid sequence substantially as set forth in SEQ ID NO:10 or having 50% amino acid similarity thereto.

40. A method according to claim 31 wherein the kinase comprises an amino acid sequence encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:9 under low stringency conditions at 42°C.

41. A method of modulating fertility in a mammal said method comprising modulating levels of HELA2 wherein increasing levels of HELA2 facilitates sperm maturation and development.

42. A method according to claim 41 wherein fertility is enhanced by introducing recombinant HELA2.

43. A method according to claim 41 wherein fertility is reduced by down regulating expression of the HELA2 gene.

44. A composition comprising a serine proteinase and/or kinase capable of regulating cell activity and/or viability and one or more pharmaceutically acceptable carriers and/or diluents.

45. A composition according to claim 44 wherein the serine proteinase is HELA2 or a functional derivative thereof.

46. An isolated antibody capable of interacting with a proteinaceous molecule involved in or associated with regulation of cell activity and/or viability comprising a sequence of amino acids encoded by a nucleotide sequence, at least a portion of which, is capable of being

amplified by polymerase chain reaction (PCR) using the following primers:

5′ ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3′ [SEQ ID NO:1]; and

5′ACAGAATTCAXIGGICCICCIC/GT/AXTCICC3′ [SEQ ID NO:2];

or a complementary form of said primers.

47. An isolated antibody according to claim 46 wherein said proteinaceous molecule is a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:4 or an amino acid sequence having at least 50% similarity thereto.

48. An isolated antibody according to claim 46 wherein said proteinaceous molecule is a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least 50% similarity thereto.

49. An isolated antibody according to claim 46 wherein said proteinaceous molecule is a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity thereto.

50. An isolated antibody according to claim 46 wherein said proteinaceous molecule is a serine proteinase comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions at 42°C.

51. An isolated antibody according to claim 46 wherein said proteinaceous molecule is a serine proteinase comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions at 42°C.

52. An isolated antibody according to claim 46 wherein said proteinaceous said molecule is a serine proteinase comprising a sequence of amino acids encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:7 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:7 under low stringency conditions at 42°C.

53. An isolated antibody according to claim 46 wherein said proteinaceous molecule is a kinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:10 or having 50% amino acid similarity thereto.

54. An isolated antibody according to claim 46 wherein said proteinaceous molecule is a kinase comprising an amino acid sequence encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:9 under low stringency conditions at 42°C.

55. An antagonist or agonist to the isolated proteinaceous molecule according to any one of claims 1 to 9.

56. A method of determining a predisposition for or the presence of a cancer, said method comprising determining the presence of a nucleotide sequence encoding a proteinaceous molecule according to any one of claims 1 to 9.

57. A method according to claim 56 wherein the nucleotide sequence encodes a polypeptide wherein at least a portion of said nucleotide sequence is capable of being amplified by polymerase chain reaction (PCR) using the following primers:

5′ ACAGAATTCTGGGTIGTIACIGCIGCICAYTG3′ [SEQ ID NO:1]; and

5′ACAGAATTCAXIGGICCICCIC/GT/AXTCICC3′ [SEQ ID NO:2];

or a complementary form of said primers.

58. A method according to claim 57 wherein said nucleotide sequence encodes a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:4 or an amino acid sequence having at least 50% similarity thereto.

59. A method according to claim 57 wherein said nucleotide sequence encodes a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least 50% similarity thereto.

60. A method according to claim 57 wherein said nucleotide sequence encodes a serine proteinase comprising an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity thereto.

61. A method according to claim 57 wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:3 or is a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions at 42°C.

62. A method according to claim 57 wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:5 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions at 42°C.

63. A method according to claim 57 wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:7 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the sequence set forth in SEQ ID NO:7 under low stringency conditions at 42°C.

64. A method according to claim 57 wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:10 or having 50% amino acid similarity thereto.

65. A method according to claim 57 wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:9 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:9 under low stringency conditions at 42°C.

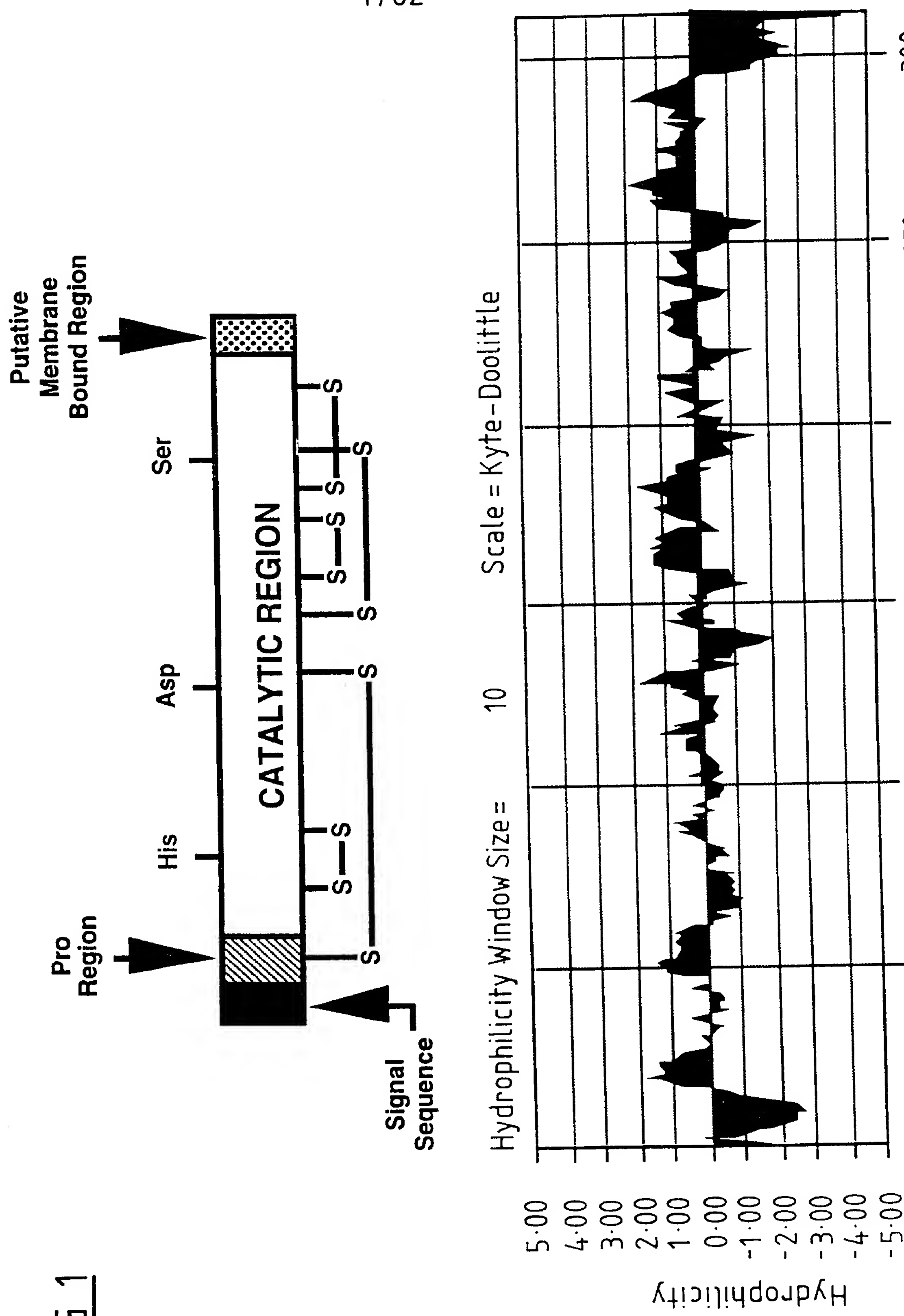
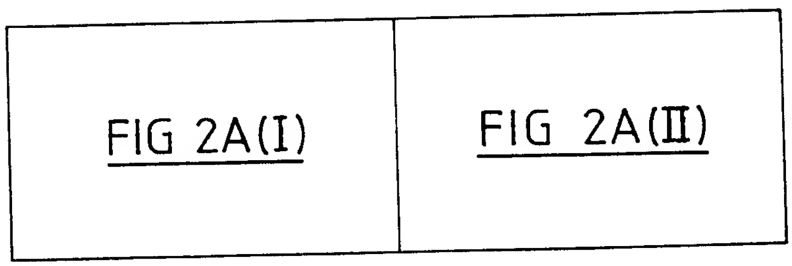

Putative Membrane Bound Region
Ser
Asp
His
Pro Region
Signal Sequence
CATALYTIC REGION
Hydrophilicity Window Size = 10 Scale = Kyte-Doolittle
Hydrophilicity
5·00
4·00
3·00
2·00
1·00
0·00
-1·00
-2·00
-3·00
-4·00
-5·00
50
100
150
200
250
300

FIG 1

FIG 2A



Sequence comparison of HELA2(Testisin) and prostasin

```
                Signal sequence                      Light Chain
prostasin  MAQKGVLGPGQLGAVAILLYLGLLRSGTGAEGA--EAPCG-VAPQARITGGSSAVA
HELA2      MGARGAL----L--LALLLARAGLRKPESQEAAPLSGPCGRRVITSRIVGGEDAEL
           *  .* *    *  .*.**    **    * *   ***     .** **  *

                 ASP                                  N-gly
                  *                            S-S      |
prostasin  KVSTLKDIIPHPSYLQEGSQGDIALLQLSRPITFSRYIRPICLPAANASFPNGLHC
HELA2      TRYFVSNIYLSPRYLG-NSPYDIALVKLSAPVTYTKHIQPICLQASTFEFENRTDC
             .  *   * **  .*  ****..**  *.*...  *.****  *..   * *  *
                                                            N-gly

       SER                         S-S
        *
prostasin  ACQGDSGGPLSCPVEGLWYLTGIVSWGDACGARNRPGVYTLASSYASWIQSKVTEL
HELA2      ACFGDSGGPLACNKDGLWYQIGVVSWGVGCGRPNRPGVYTNISHHFEWIQ------
           ** *******.*   .****  *.****   **  ******* *    ***
                     S-S                         N-gly
```

FIG 2A(I)

Sequence comparison of HELA2(Testisin) and prostasin

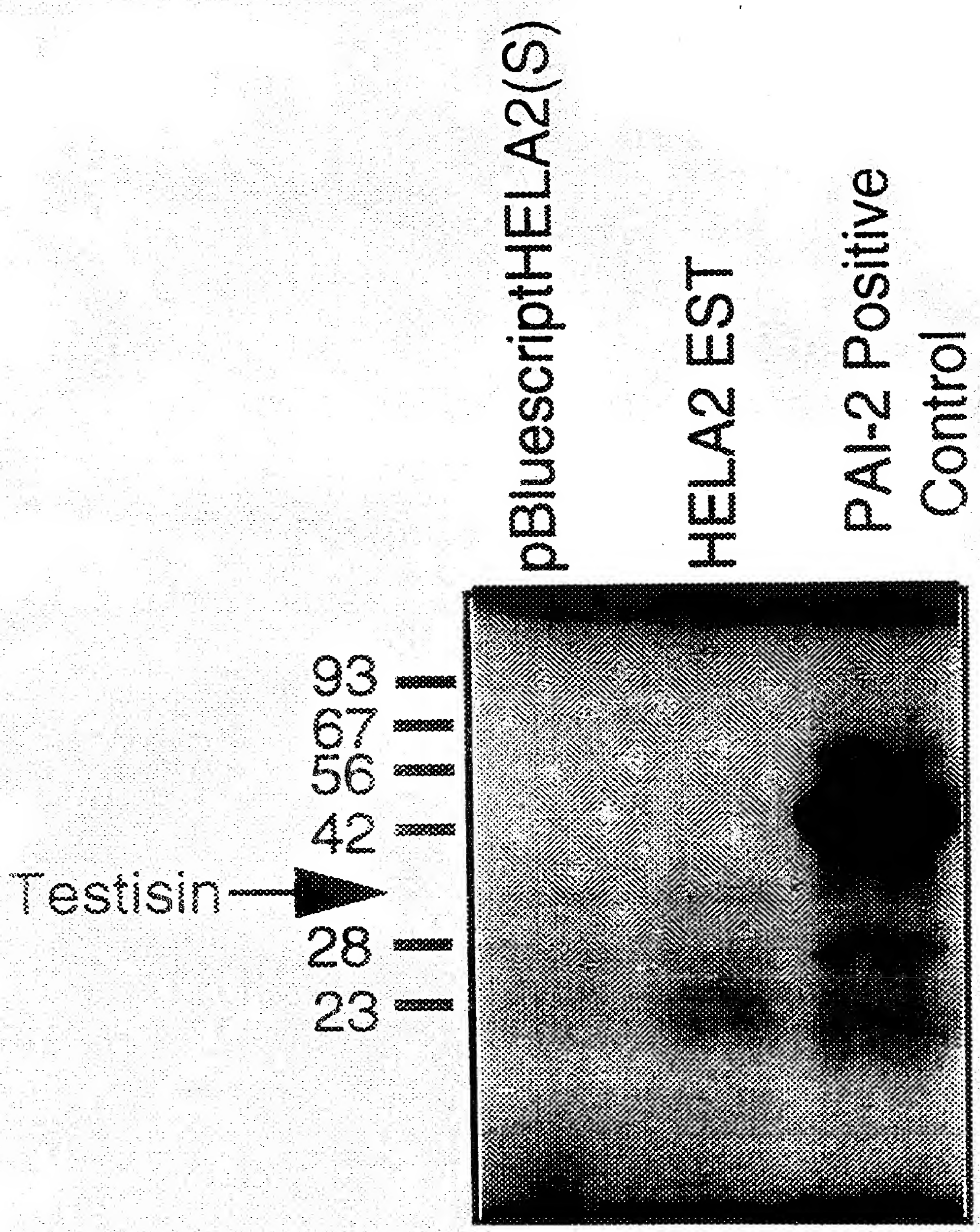
```
                                                 HIS
                                                  *
prostasin   GQWPWQVSITYEGVHVCGGSLVSEQWVLSAAHCFPSEHHK-EAYEVKLG----AHQLDSYSEDA
HELA2       GRWPWQGSLRLWDSHVCGVSLLSHRWALTAAHCFETDLSDPSGWMVQFGQLTSMPSFWSLQAYY
            *.**** *.        **** **.* .* *.*****  .        *.  *         *
                             |_______S-S_______|    YS
                                                (Long Isoform)

                         Heavy Chain
prostasin   TVTGWGHVAPSVSLLTPKPLQQLEVPLISRETCNCLYNIDAKPEEPHFVQEDMVCAGYVEGGKD
HELA2       WVTGWGYIKEDEALPSPHTLQEVQVAIINNSMCNHLFLKYSFRKD--IFG-DMVCAGNAQGGKD
             *****.     .* .*. **...* .*|  ** *.   .   .      ******  .****
                                      N-gly
                                     |__________S-S__________|

                                                   Putative
                                                   Transmembrane
                                                   Domain
prostasin   QPRVVPQTQESQPDSNLCGSHLAFSSAPAQGLLR     PILFLPLGLALGLLSPWL  SEH
HELA2       --KLMAQSGMSQPD-------------PS----W     PLLFFPLLWALPLLGPV
              ... *.  ****             *.          *.** **  ** ** *
```

FIG 2A(II)

FIG 2B



In vitro transcription / translation of HELA2 (Testisin)

FIG 3

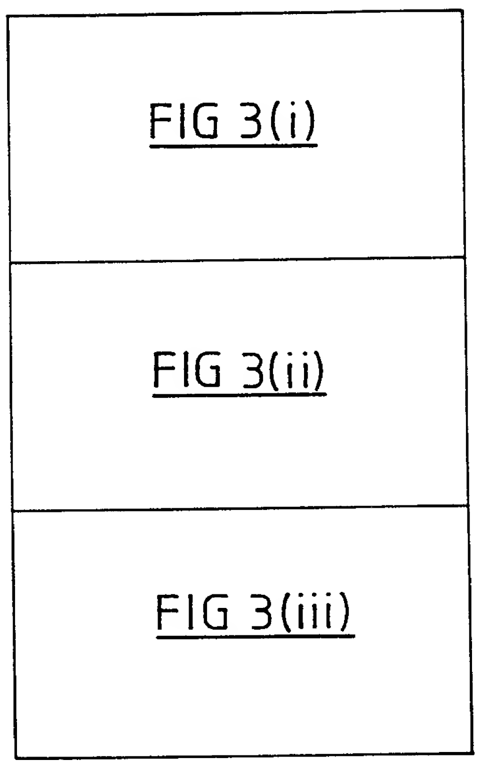
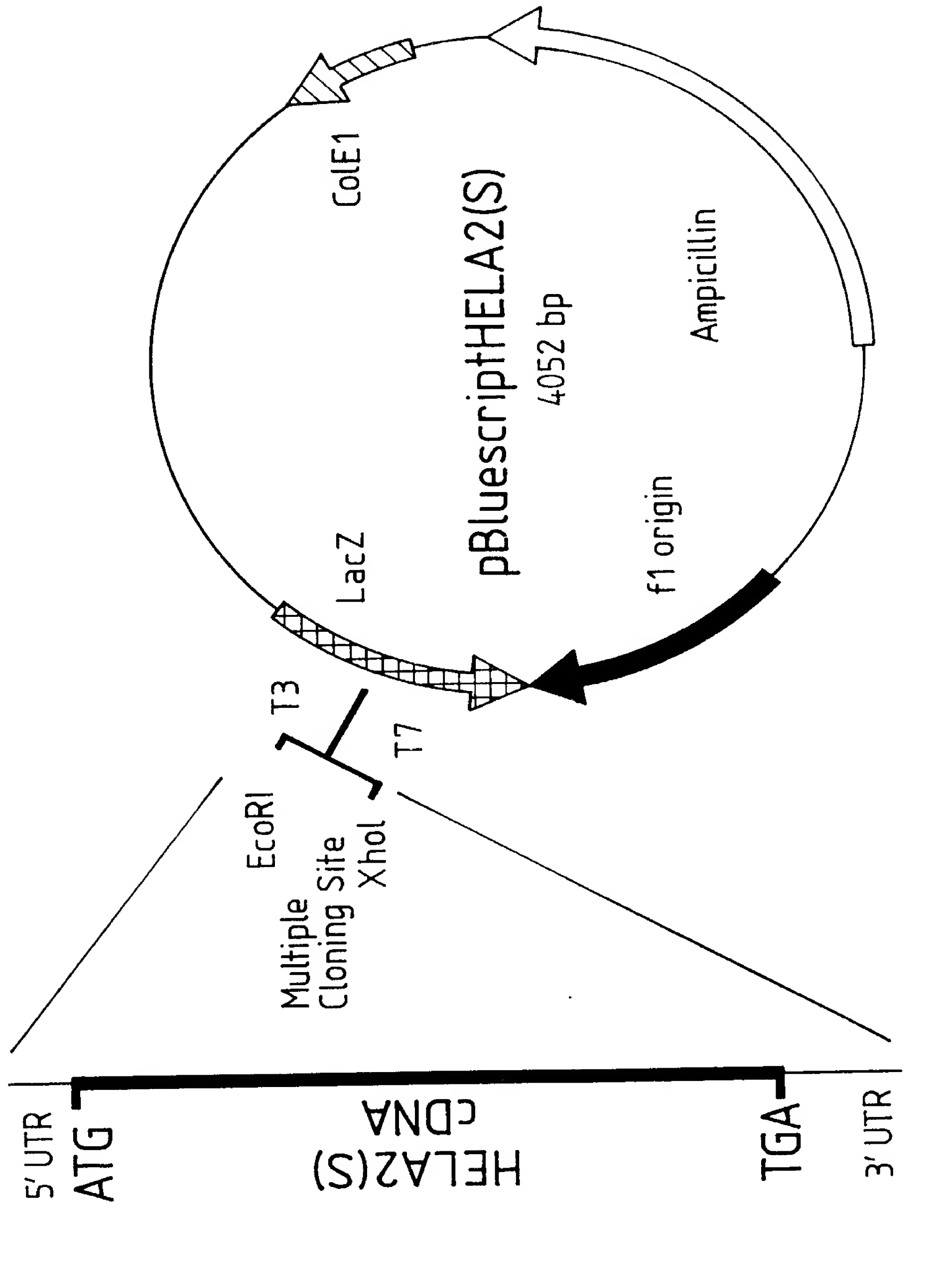
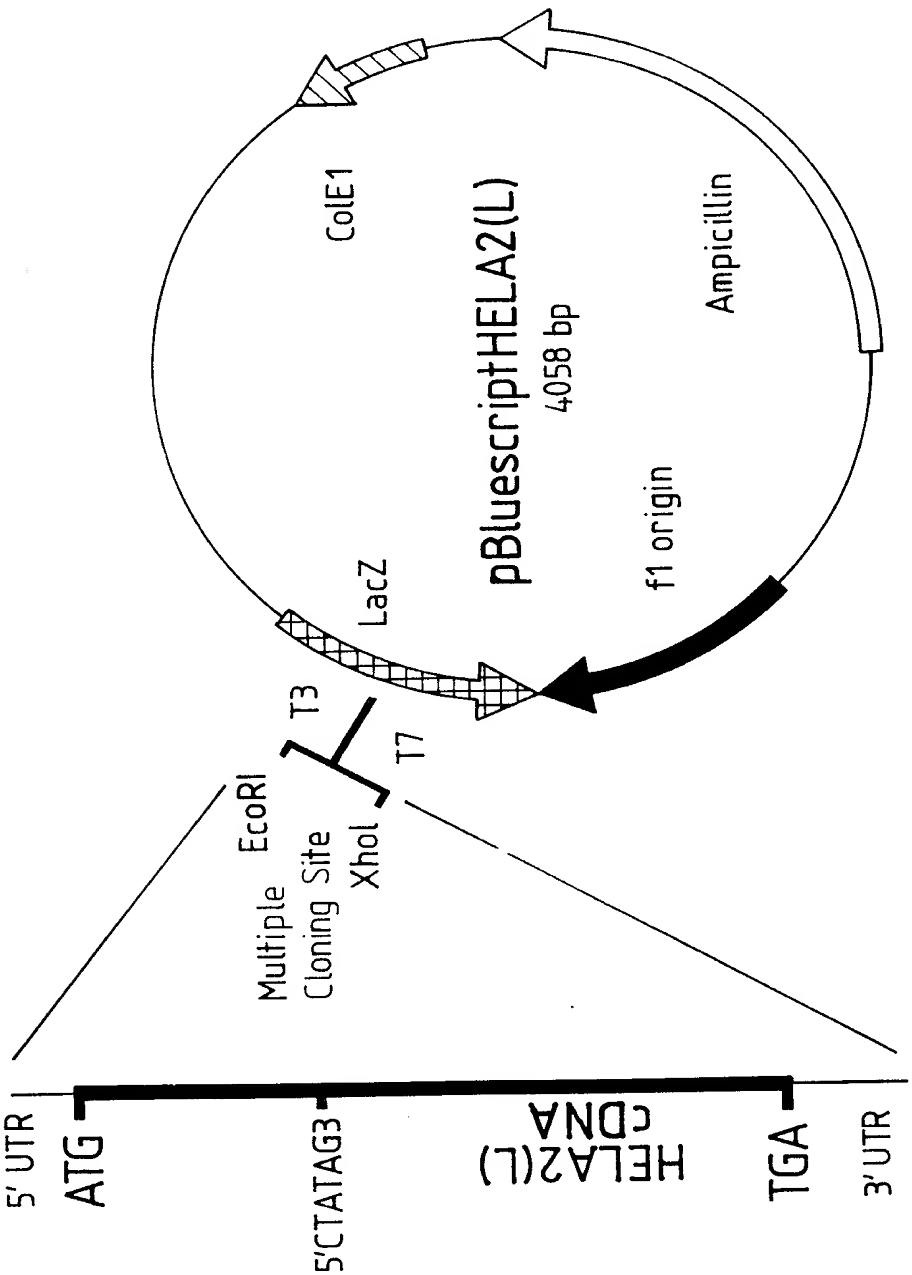


FIG 3(i)



HELA2 (Testisin) Short Isoform



HELA2 (Testisin) Long Isoform

FIG 3(ii)

HELA2 (Testisin) Restriction Enzyme Map

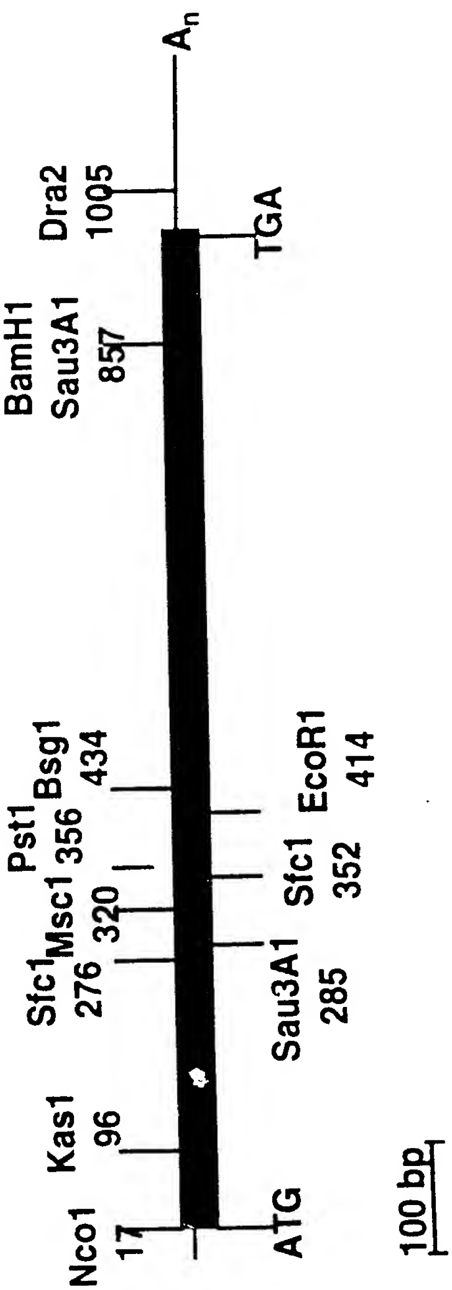
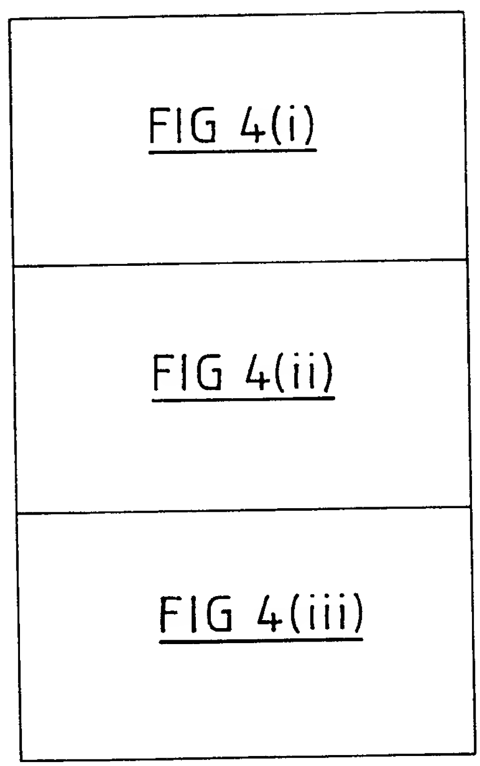


FIG 3(iii)

FIG 4



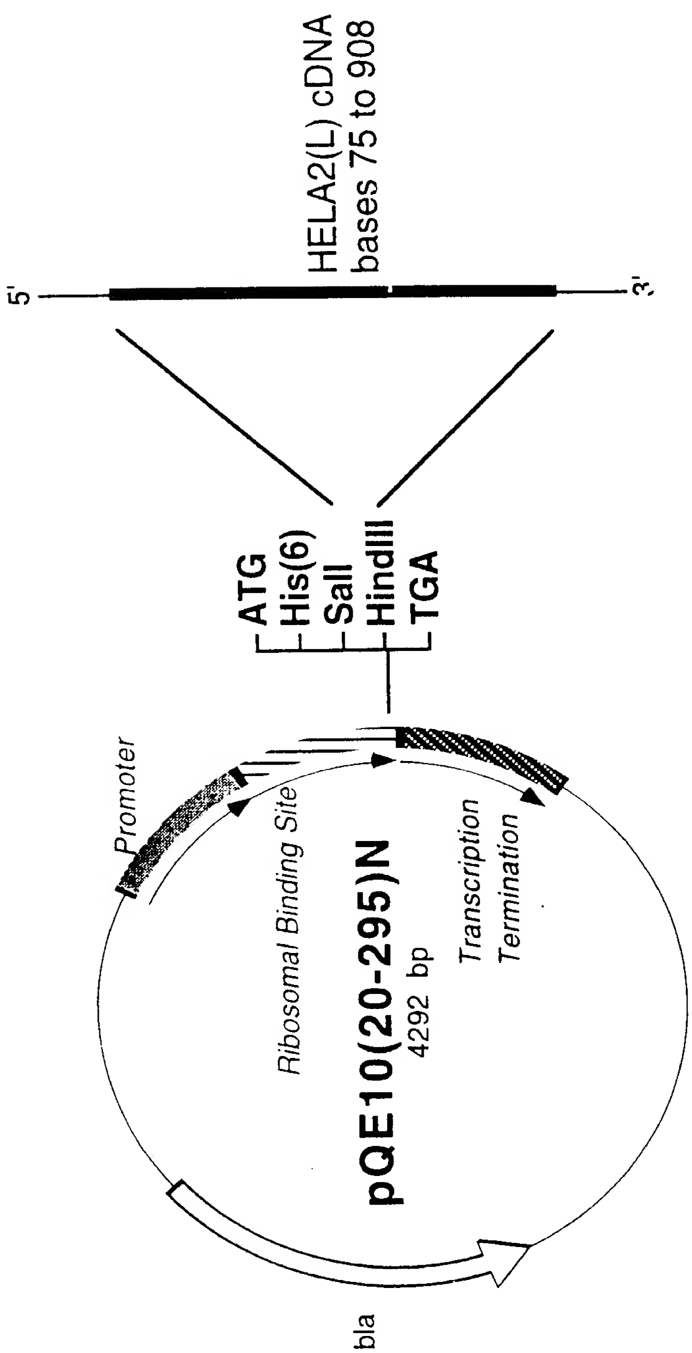

HELA2(L) cDNA
bases 75 to 908
5'
3'
ATG
His(6)
SalI
HindIII
TGA
Promoter
Ribosomal Binding Site
Transcription
Termination
pQE10(20-295)N
4292 bp
bla

FIG 4(i)

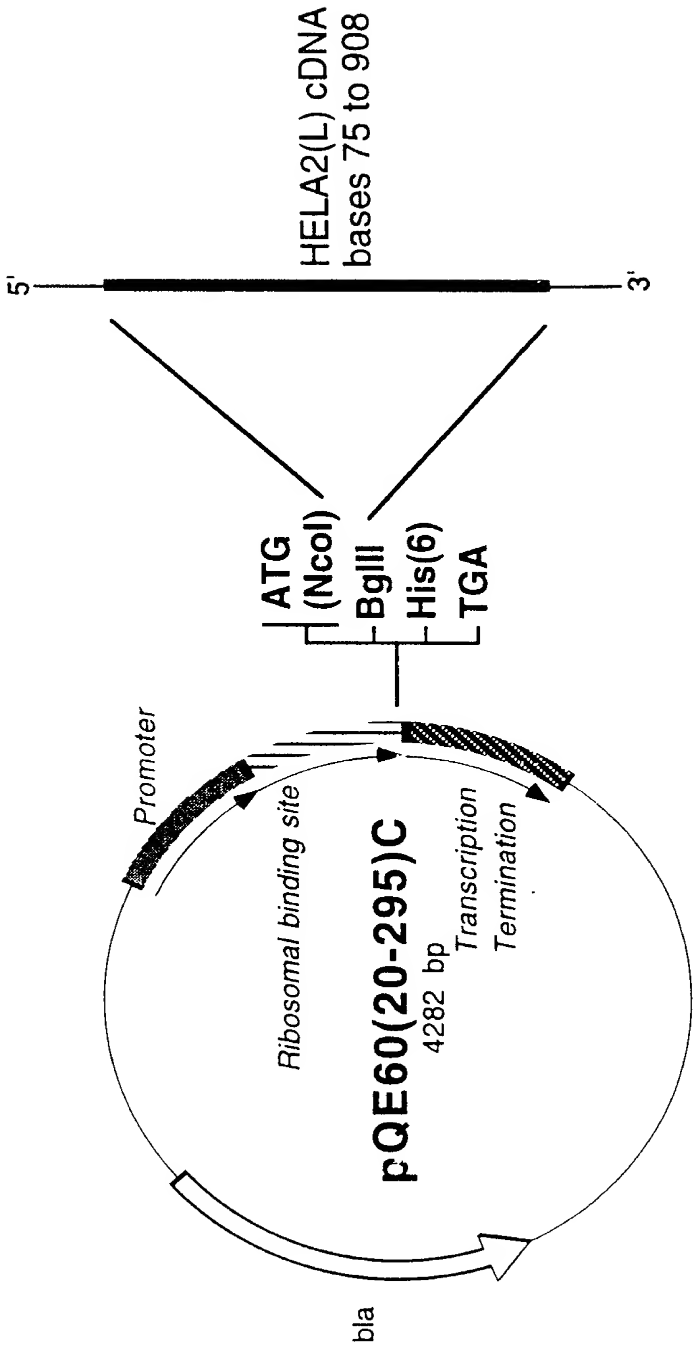

HELA2(L) cDNA
bases 75 to 908
5'
3'
ATG
(NcoI)
BglII
His(6)
TGA
Promoter
Ribosomal binding site
pQE60(20-295)C
4282 bp
Transcription
Termination
bla

FIG 4(ii)

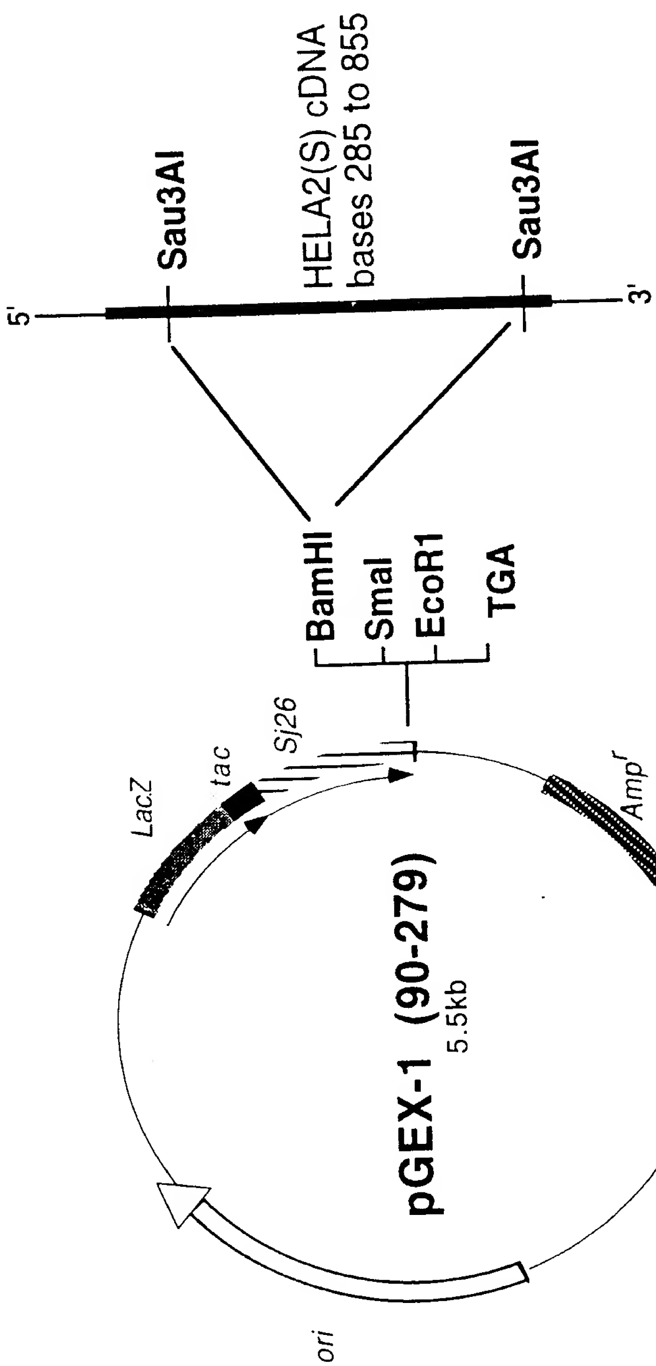
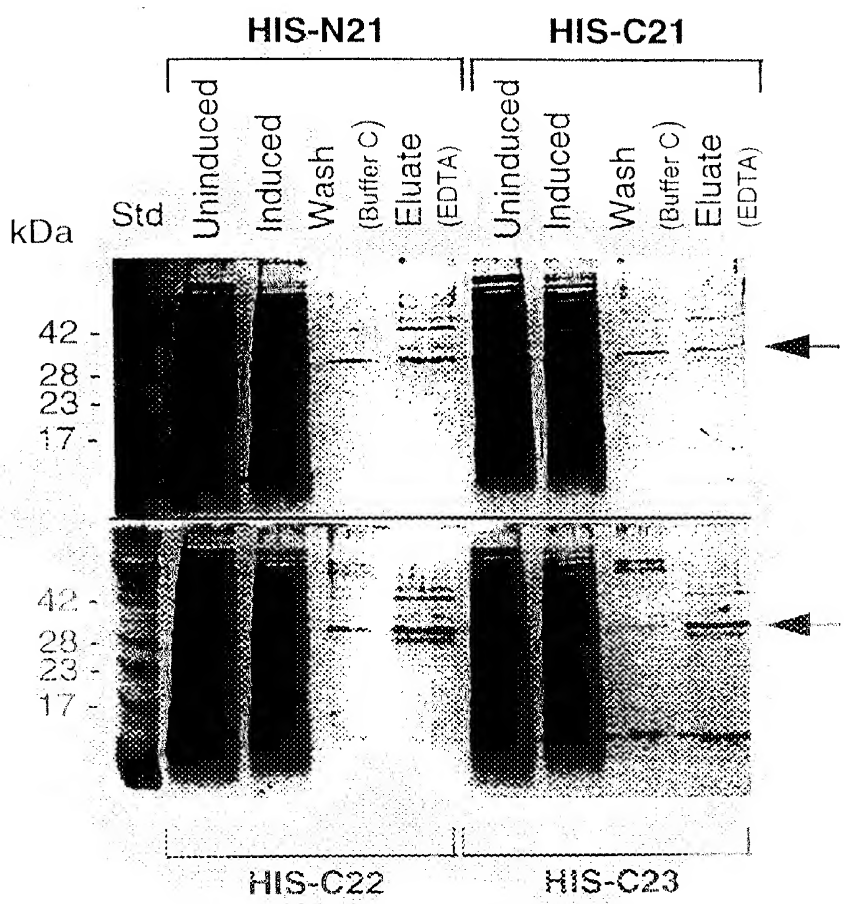

5'
Sau3AI
HELA2(S) cDNA
bases 285 to 855
Sau3AI
3'
BamHI
SmaI
EcoR1
TGA
LacZ
tac
Sj26
Amp^r
pGEX-1 (90-279)
5.5kb
ori

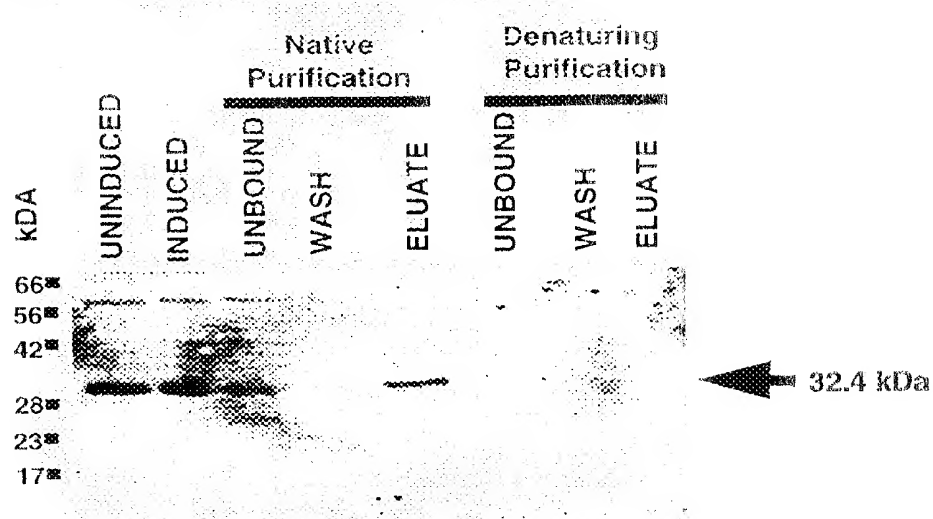
FIG 4(iii)

FIG 5

A. Expression of recombinant Testisin in *E. coli*.



B. Western blot of recombinant Testisin



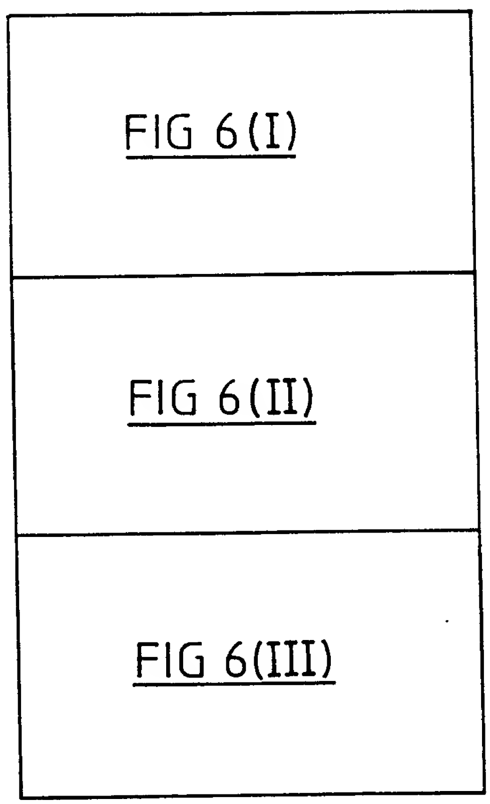


FIG 6

FIGURE 6(I)

```
1                                                                  GCCGCGGGAGAGGAGGCC
19   ATGGGCGCGCGGGGCGCTGCTGCTGGCGCTGCTGCTGGCTCGGGCTGGACTCAGGAAG
      M  G  A  R  G  A  L  L  L  A  L  L  L  A  R  A  G  L  R  K    20

79   CCGGAGTCGCAGGAGGCGGCGCCGTTATCAGGACCATGCGGCCGACGGGTCATCACGTCG
      P  E  S  Q  E  A  A  P  L  S  G  P  C  G  R  R  V  I  T  S    40

139  CGCATCGTGGGTGGAGAGGACGCCGAACTCGGGCGTTGGCCGTGGCAGGGGAGCCTGCGC
      R  I  V  G  G  E  D  A  E  L  G  R  W  P  W  Q  G  S  L  R    60

199  CTGTGGGATTCCCACGTATGCGGAGTGAGCCTGCTCAGCCACCGCTGGGCACTCACGGCG
      L  W  D  S  H  V  C  G  V  S  L  L  S  H  R  W  A  L  T  A    80

259  GCGCACTGCTTTGAAACCTATAGTGACCTTAGTGATCCCTCCGGGTGGATGGTCCAGTTT
      A  H  C  F  E  T  Y  S  D  L  S  D  P  S  G  W  M  V  Q  F   100

319  GGCCAGCTGACTTCCATGCCATCCTTCTGGAGCCTGCAGGCCTACTACACCCGTTACTTC
      G  Q  L  T  S  M  P  S  F  W  S  L  Q  A  Y  Y  T  R  Y  F   120

379  GTATCGAATATCTATCTGAGCCCTCGCTACCTGGGGAATTCACCCTATGACATTGCCTTG
      V  S  N  I  Y  L  S  P  R  Y  L  G  N  S  P  Y  D  I  A  L   140
```

FIGURE 6 (II)

```
439  GTGAAGCTGTCTGCACCTGTCACCTACACTAAACACATCCAGCCCATCTGTCTCCAGGCC
      V  K  L  S  A  P  V  T  Y  T  K  H  I  Q  P  I  C  L  Q  A   160

499  TCCACATTTGAGTTTGAGAACCGGACAGACTGCTGGGTGACTGGCTGGGGGTACATCAAA
      S  T  F  E  F  E  N  R  T  D  C  W  V  T  G  W  G  Y  I  K   180

559  GAGGATGAGGCACTGCCATCTCCCCACACCCTCCAGGAAGTTCAGGTCGCCATCATAAAC
      E  D  E  A  L  P  S  P  H  T  L  Q  E  V  Q  V  A  I  I  N   200

619  AACTCTATGTGCAACCACCTCTTCCTCAAGTACAGTTTCCGCAAGGACATCTTTGGAGAC
      N  S  M  C  N  H  L  F  L  K  Y  S  F  R  K  D  I  F  G  D   220

679  ATGGTTTGTGCTGGCAATGCCCAAGGCGGGAAGGATGCCTGCTTCGGTGACTCAGGTGGA
      M  V  C  A  G  N  A  Q  G  G  K  D  A  C  F  G  D  S  G  G   240

739  CCCTTGGCCTGTAACAAGAATGGACTGTGGTATCAGATTGGAGTCGTGAGCTGGGGAGTG
      P  L  A  C  N  K  N  G  L  W  Y  Q  I  G  V  V  S  W  G  V   260

799  GGCTGTGGTCGGCCCAATCGGCCCGGTGTCTACACCAATATCAGCCACCACTTTGAGTGG
      G  C  G  R  P  N  R  P  G  V  Y  T  N  I  S  H  H  F  E  W   280
```

FIGURE 6 (III)

```
859    ATCCAGAAGCTGATGGCCCAGAGTGGCATGTCCCAGCCAGACCCCTCCTGGCCGCTACTC
        I  Q  K  L  M  A  Q  S  G  M  S  Q  P  D  P  S  W  P  L  L  300

919    TTTTTCCCTCTTCTCTGGGCTCTCCCACTCCTGGGGCCGGTCTGAGCCTACCTGAGCCCA 314
        F  F  P  L  L  W  A  L  P  L  L  G  P  V  *

979    TGCAGCCTGGGGCCACTGCCAAGTCAGGCCCTGGTTCTCTTCTGTCTTGTTTGGTAATAA
1039   ACACATTCCAGTTGATGCCTTGCAGGCATTCTTCAAAAAAAAAAAAAAAAAAAAAAAAAA
1099   AAAAAAAAAAAAAAAAAAA
```

Western blot of GST-Testisin using anti-Testisin peptide T175 antibody

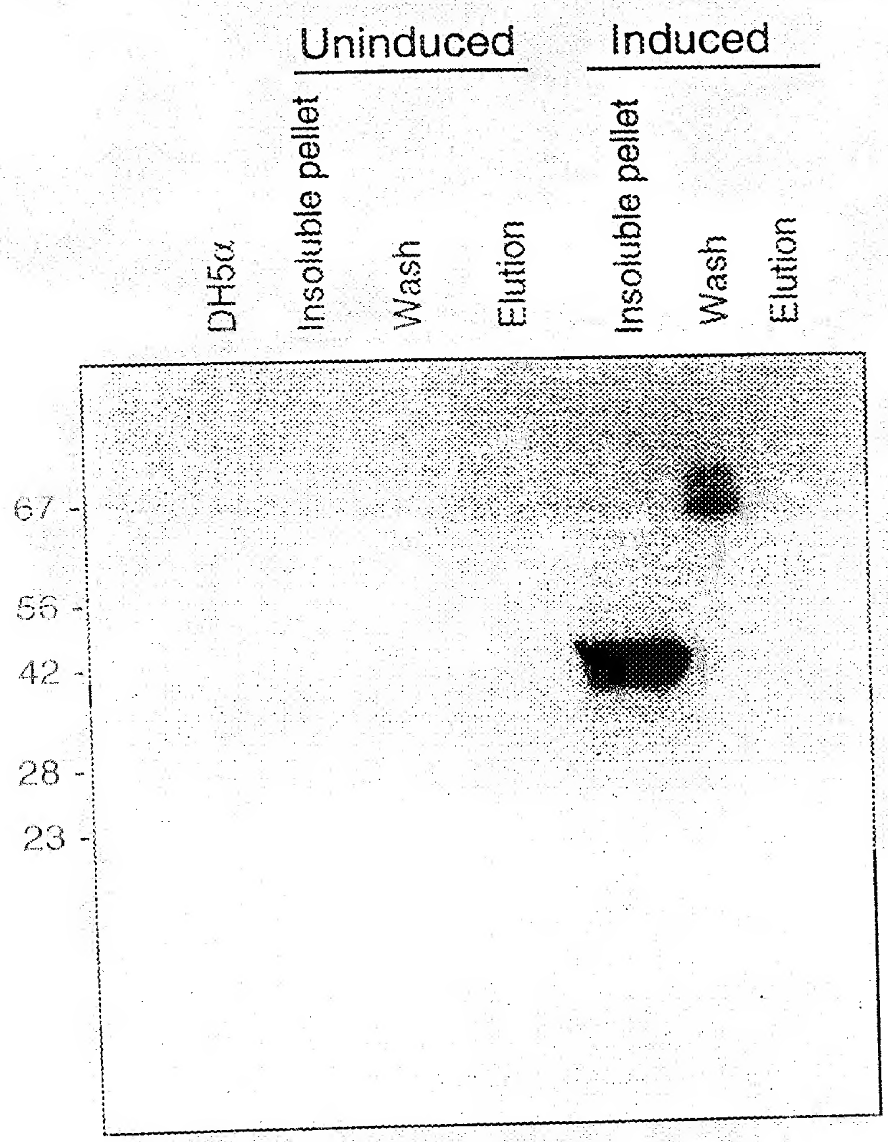
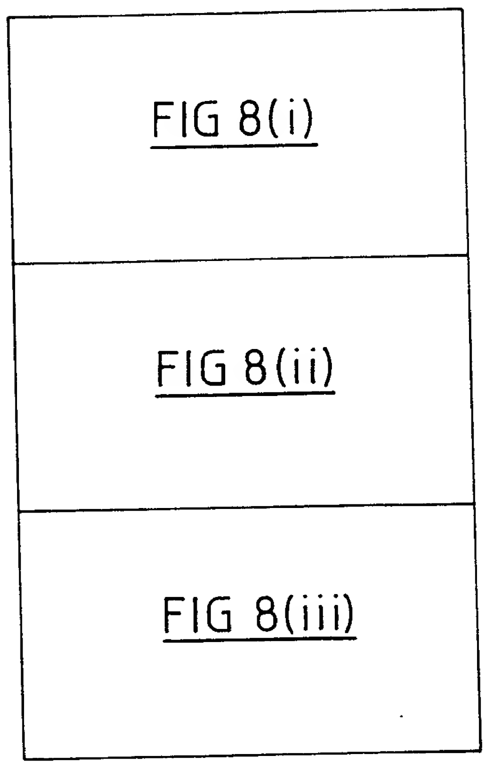


FIG 7

FIG 8



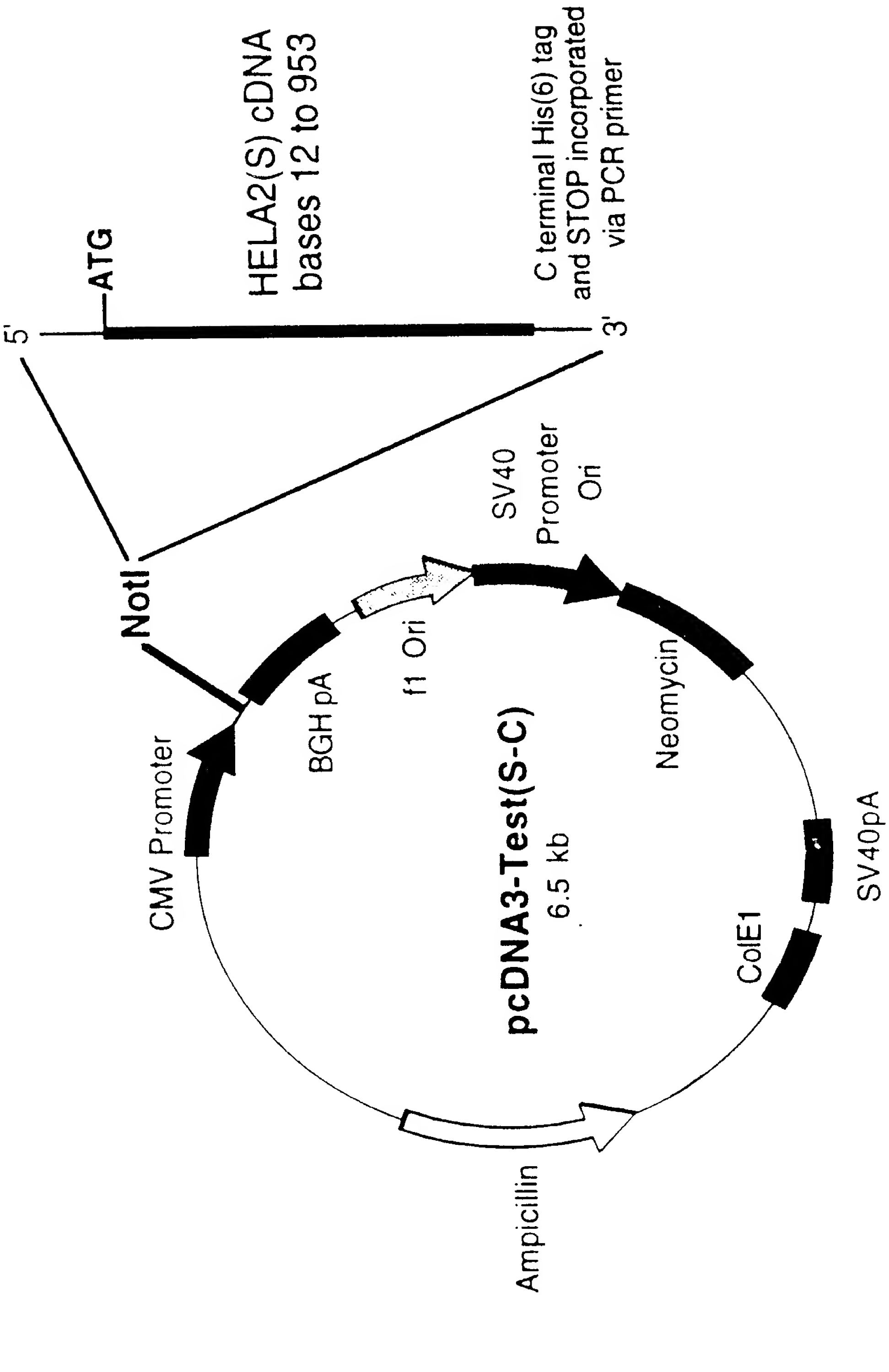

HELA2(S) cDNA
bases 12 to 953
ATG
5'
3'
C terminal His(6) tag
and STOP incorporated
via PCR primer
NotI
SV40
Promoter
Ori
f1 Ori
BGH pA
CMV Promoter
pcDNA3-Test(S-C)
6.5 kb
Neomycin
SV40pA
ColE1
Ampicillin

FIG 8(i)

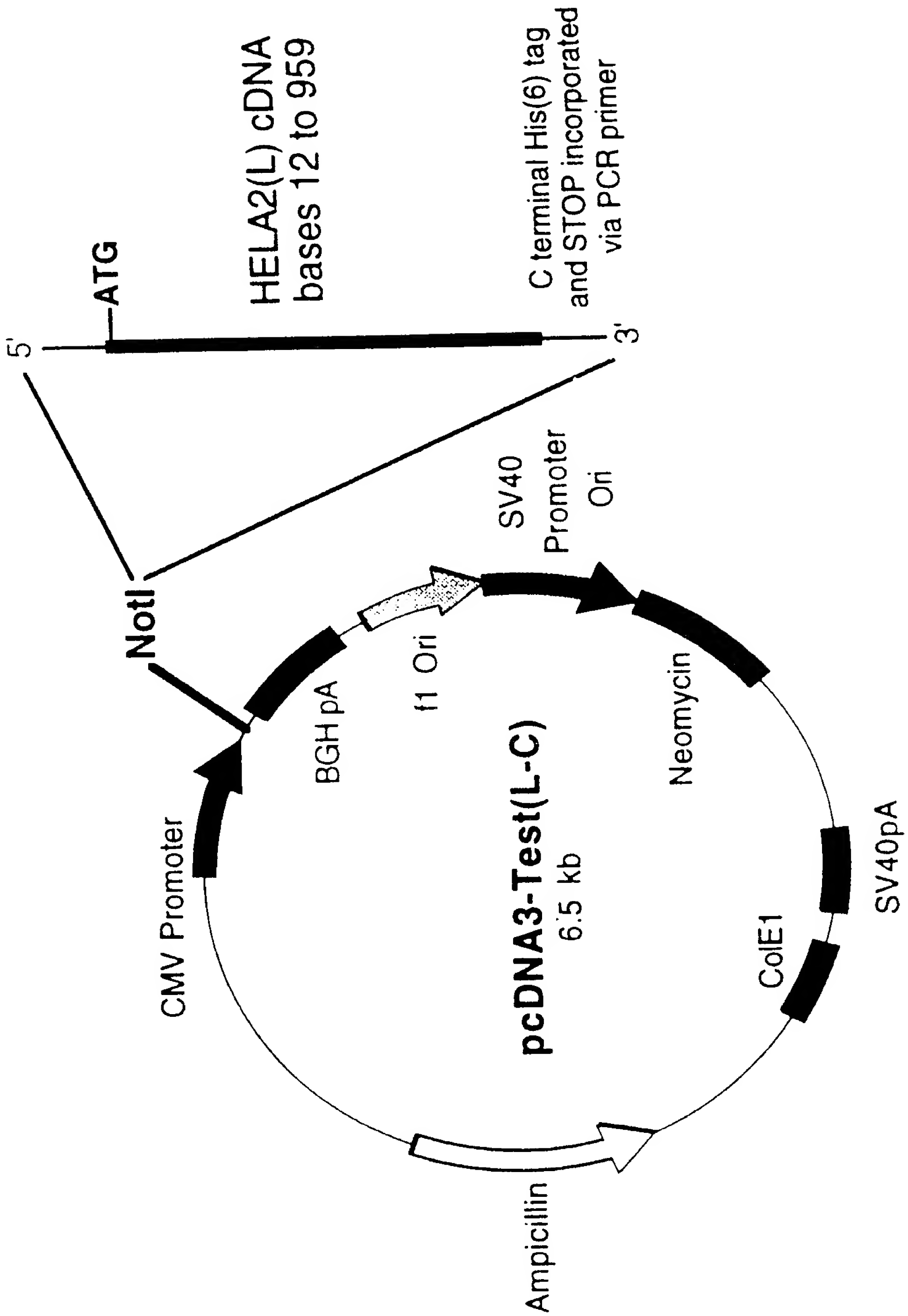

HELA2(L) cDNA
bases 12 to 959
ATG
5'
3'
C terminal His(6) tag
and STOP incorporated
via PCR primer
NotI
SV40
Promoter
Ori
CMV Promoter
BGH pA
f1 Ori
Neomycin
pcDNA3-Test(L-C)
6.5 kb
SV40pA
ColE1
Ampicillin

FIG 8(ii)

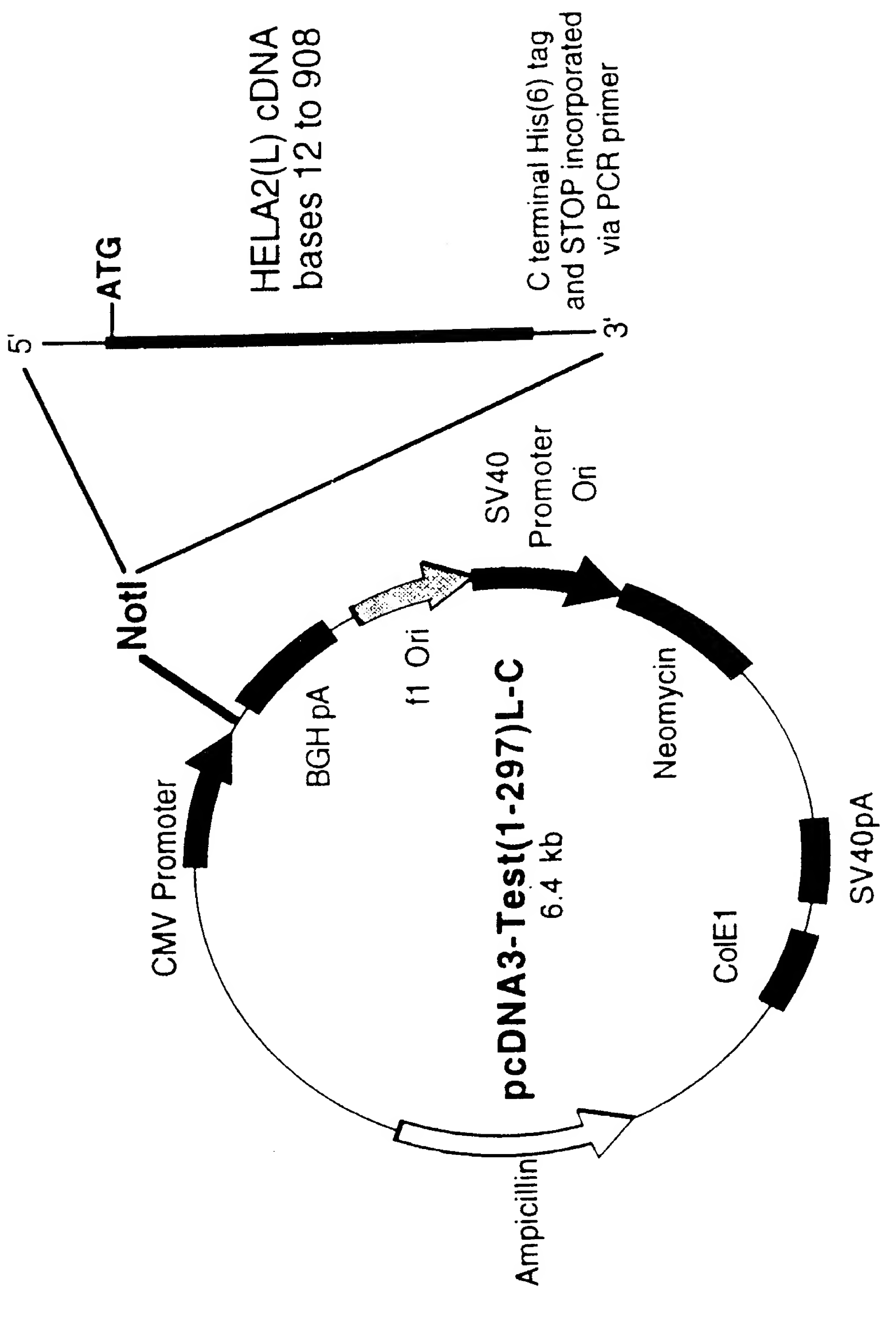

NotI
5'
ATG
3'
HELA2(L) cDNA
bases 12 to 908
C terminal His(6) tag
and STOP incorporated
via PCR primer
CMV Promoter
BGH pA
f1 Ori
SV40
Promoter
Ori
Neomycin
SV40pA
ColE1
Ampicillin
pcDNA3-Test(1-297)L-C
6.4 kb

FIG 8(iii)

FIG 9

FIG 9(i)	FIG 9(ii)
FIG 9(iii)	FIG 9(iv)

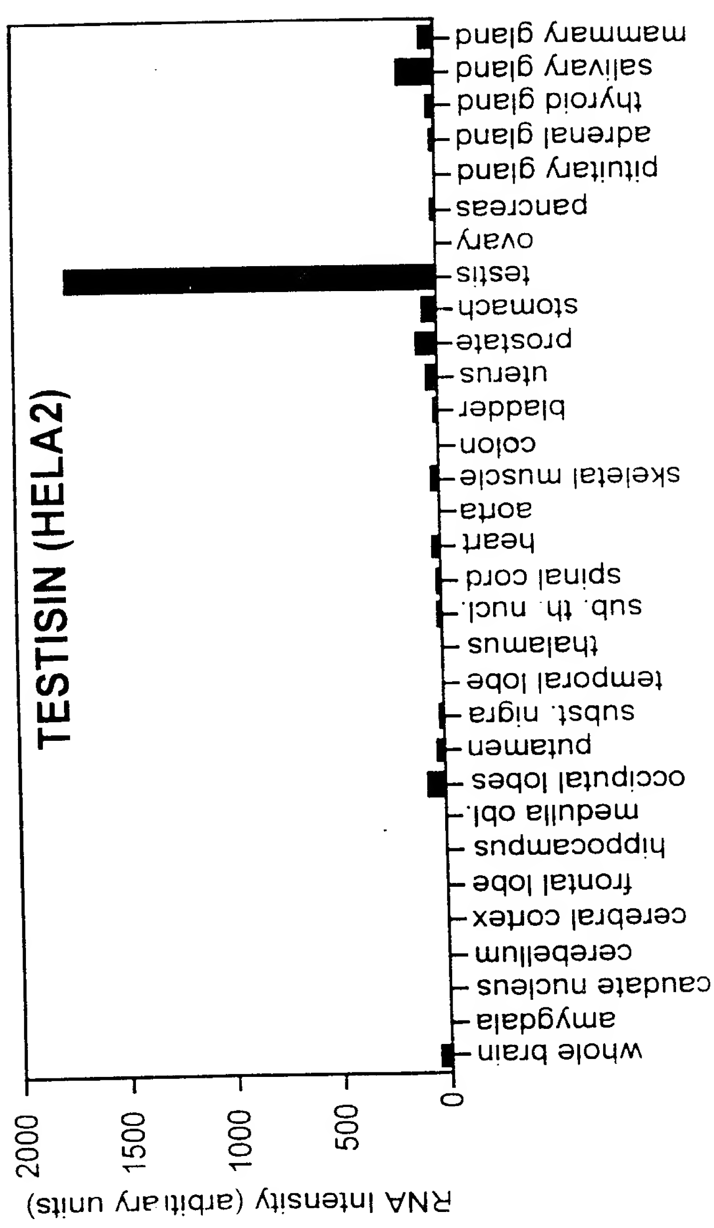

TESTISIN (HELA2)
RNA intensity (arbitrary units)
2000
1500
1000
500
0
whole brain
amygdala
caudate nucleus
cerebellum
cerebral cortex
frontal lobe
hippocampus
medulla obl.
occipital lobes
putamen
subst. nigra
temporal lobe
thalamus
sub. th. nucl.
spinal cord
heart
aorta
skeletal muscle
colon
bladder
uterus
prostate
stomach
testis
ovary
pancreas
pituitary gland
adrenal gland
thyroid gland
salivary gland
mammary gland

FIG 9(i)

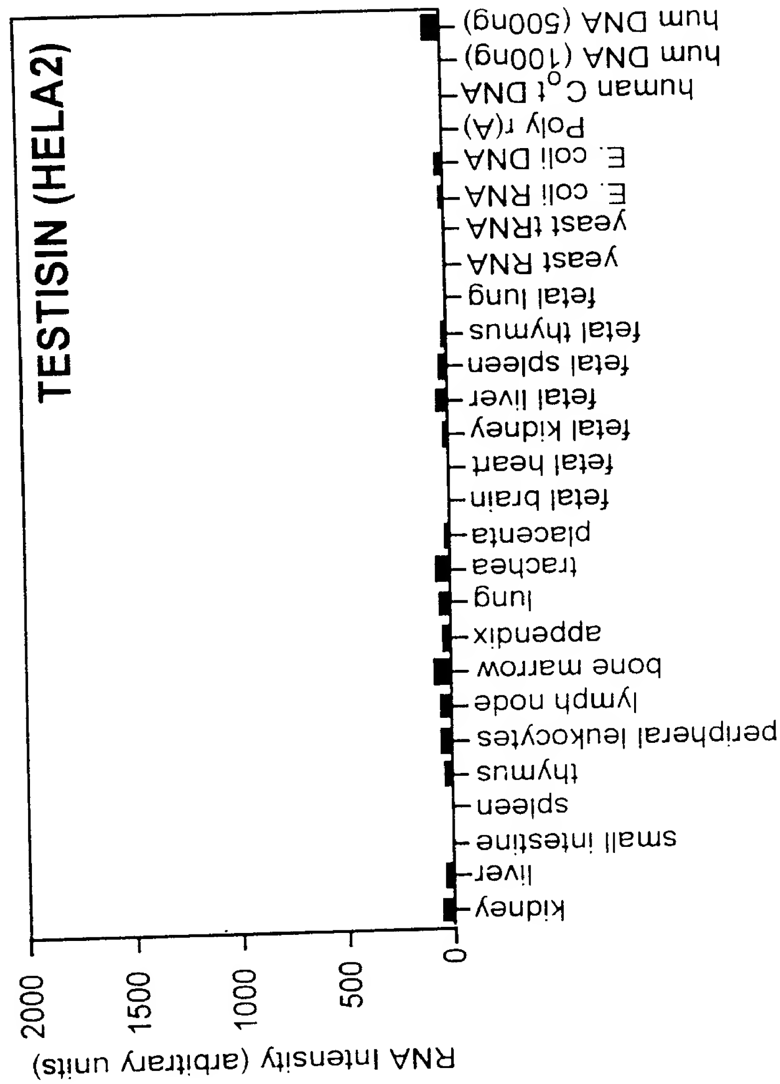

TESTISIN (HELA2)
RNA Intensity (arbitrary units)
0
500
1000
1500
2000
kidney
liver
small intestine
spleen
thymus
peripheral leukocytes
lymph node
bone marrow
appendix
lung
trachea
placenta
fetal brain
fetal heart
fetal kidney
fetal liver
fetal spleen
fetal thymus
fetal lung
yeast RNA
yeast tRNA
E. coli RNA
E. coli DNA
Poly r(A)
human C_0t DNA
hum DNA (100ng)
hum DNA (500ng)

FIG 9(ii)

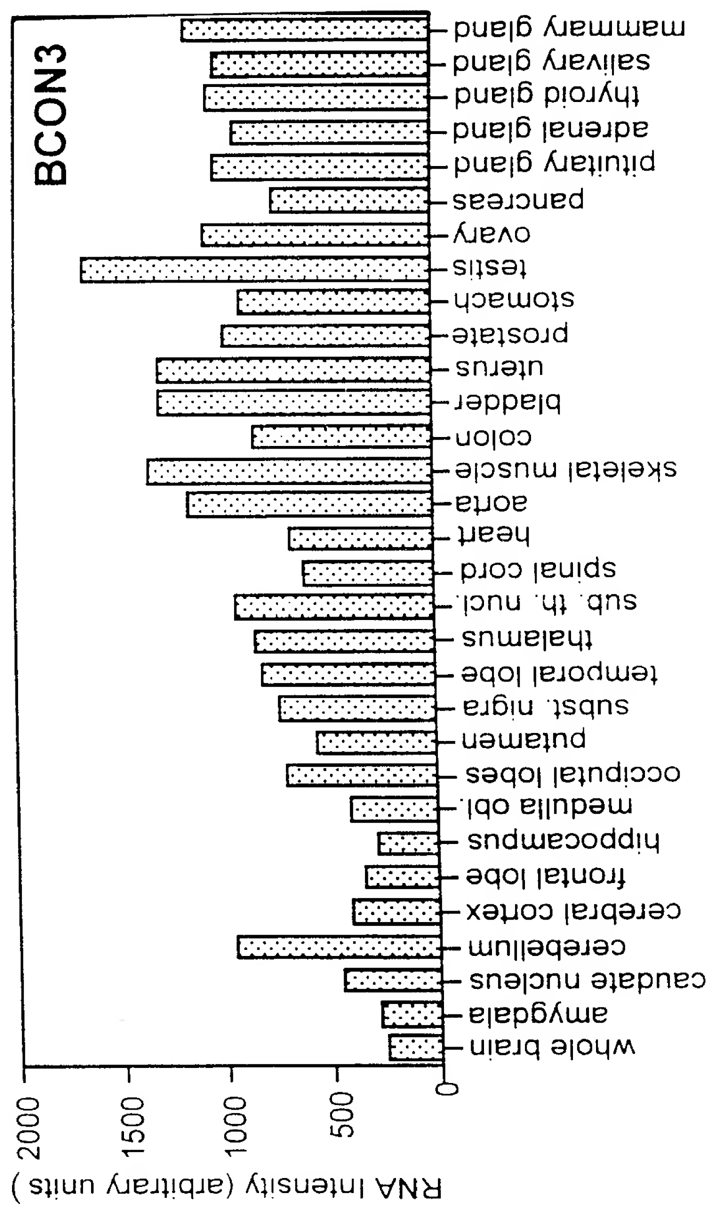

BCON3
RNA Intensity (arbitrary units)
0
500
1000
1500
2000
whole brain
amygdala
caudate nucleus
cerebellum
cerebral cortex
frontal lobe
hippocampus
medulla obl.
occiputal lobes
putamen
subst. nigra
temporal lobe
thalamus
sub. th. nucl.
spinal cord
heart
aorta
skeletal muscle
colon
bladder
uterus
prostate
stomach
testis
ovary
pancreas
pituitary gland
adrenal gland
thyroid gland
salivary gland
mammary gland

FIG 9(iii)

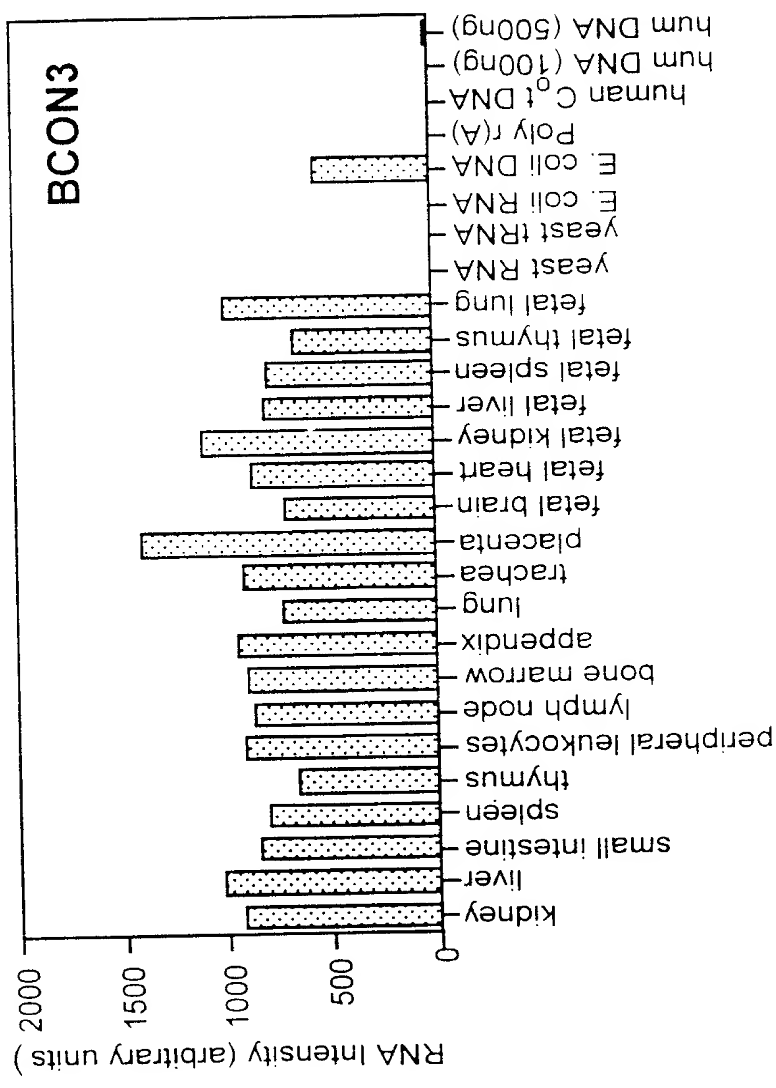

BCON3
RNA Intensity (arbitrary units)
2000
1500
1000
500
0
kidney
liver
small intestine
spleen
thymus
peripheral leukocytes
lymph node
bone marrow
appendix
lung
trachea
placenta
fetal brain
fetal heart
fetal kidney
fetal liver
fetal spleen
fetal thymus
fetal lung
yeast RNA
yeast tRNA
E. coli RNA
E. coli DNA
Poly r(A)
human C_ot DNA
hum DNA (100ng)
hum DNA (500ng)

FIG 9(iv)

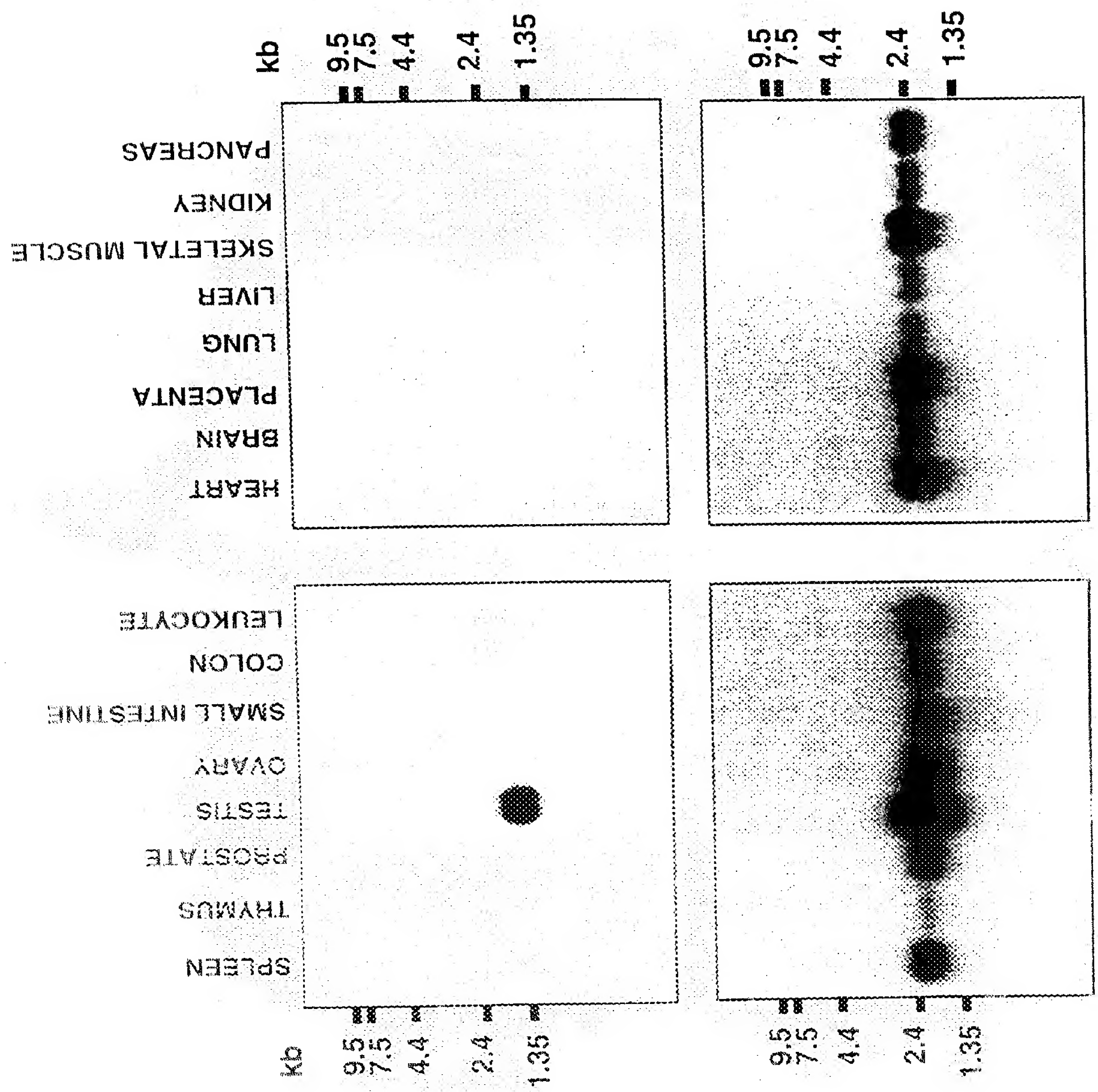


FIG 10

A. HELA2 (Testisin)

B. BCON3

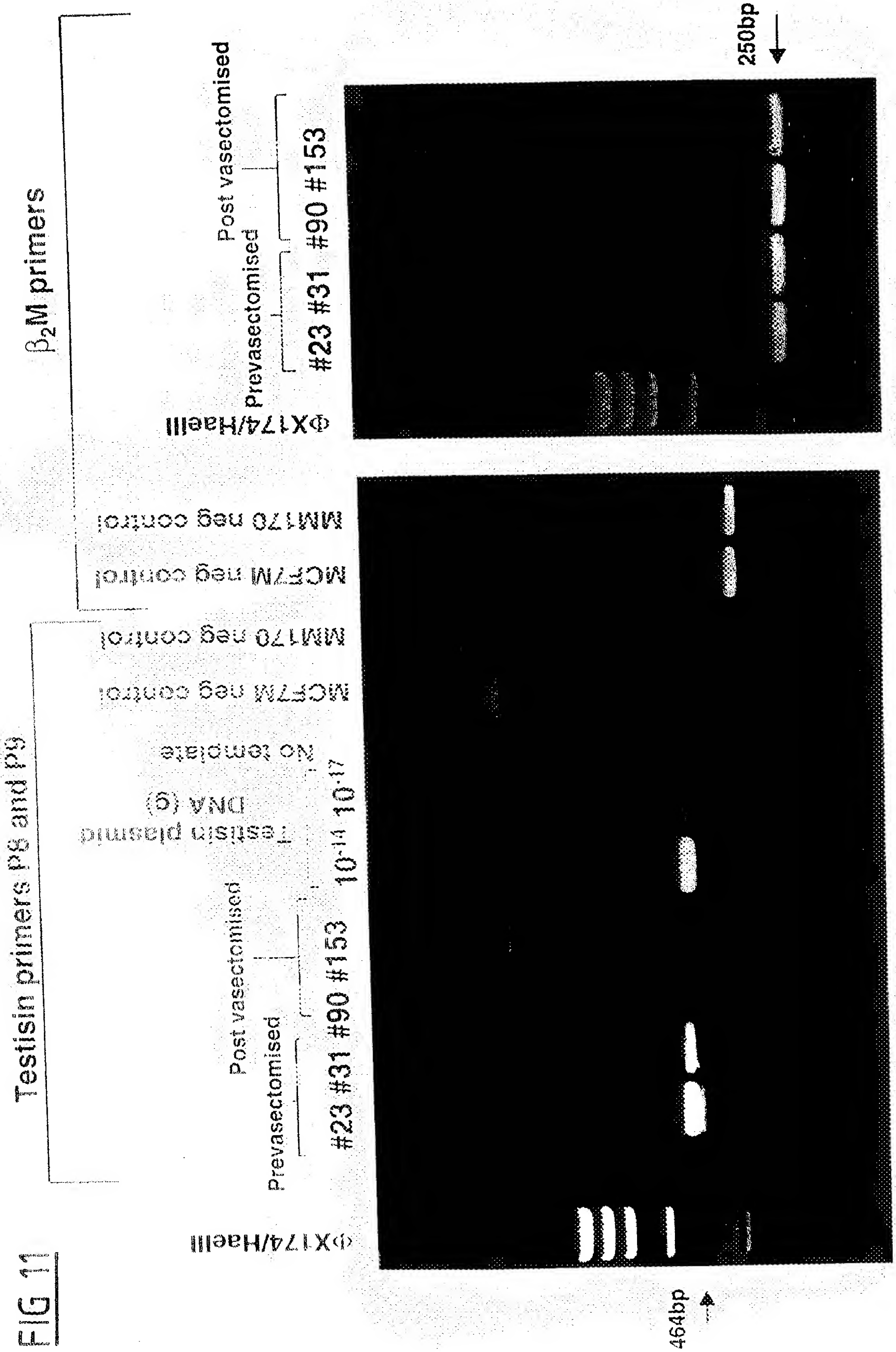

β2M primers
Post vasectomised
Prevasectomised
#23 #31 #90 #153
ΦX174/HaeIII
250bp
MM170 neg control
MCF7M neg control
Testisin primers P8 and P9
No template
Testisin plasmid DNA (g)
10-14 10-17
464bp

FIG 11

FIG 12

Testisin (HELA2) is located on human chromosome 16p13.3

A

FIG 13A

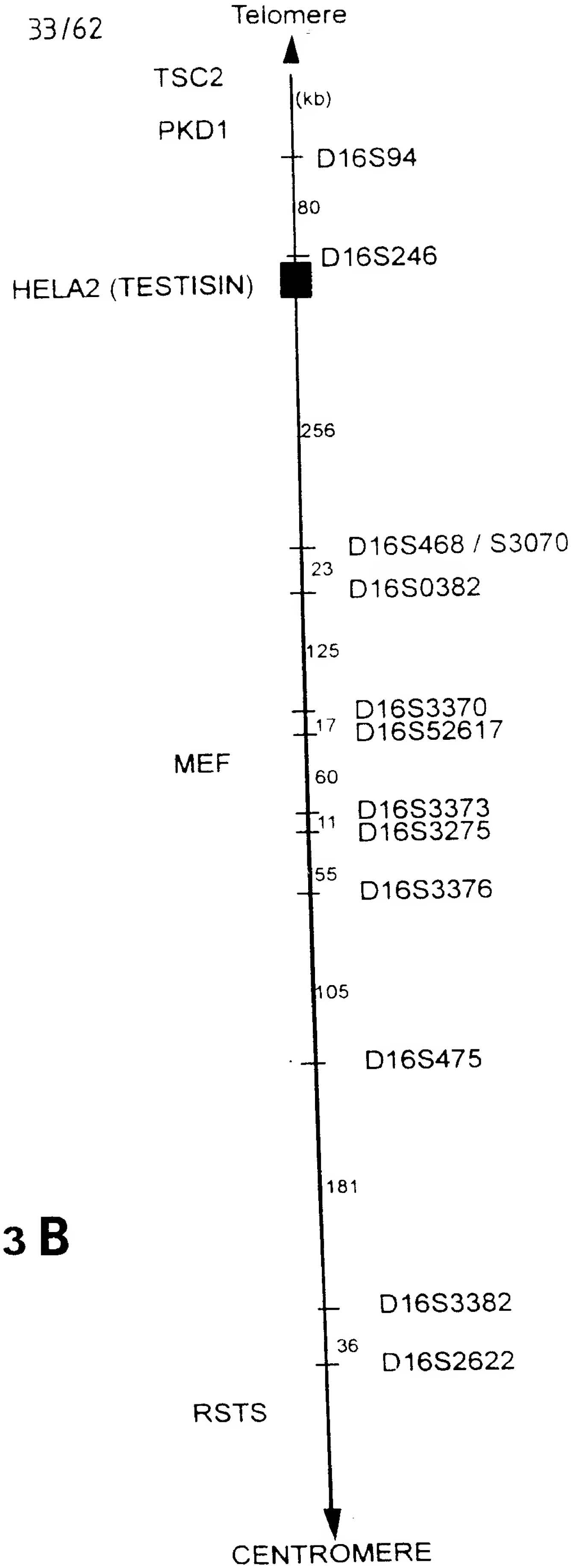
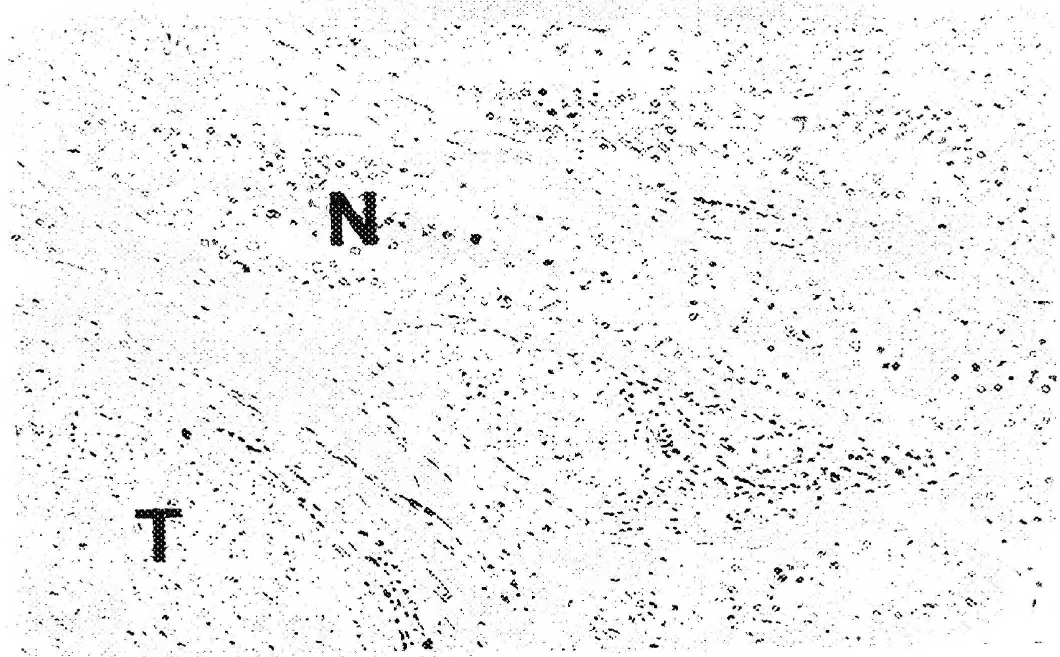

Telomere
TSC2
(kb)
PKD1
D16S94
80
D16S246
HELA2 (TESTISIN)
256
D16S468 / S3070
23
D16S0382
125
D16S3370
17
D16S52617
MEF
60
D16S3373
11
D16S3275
55
D16S3376
105
D16S475
181
D16S3382
36
D16S2622
RSTS
CENTROMERE

FIGURE 13 B

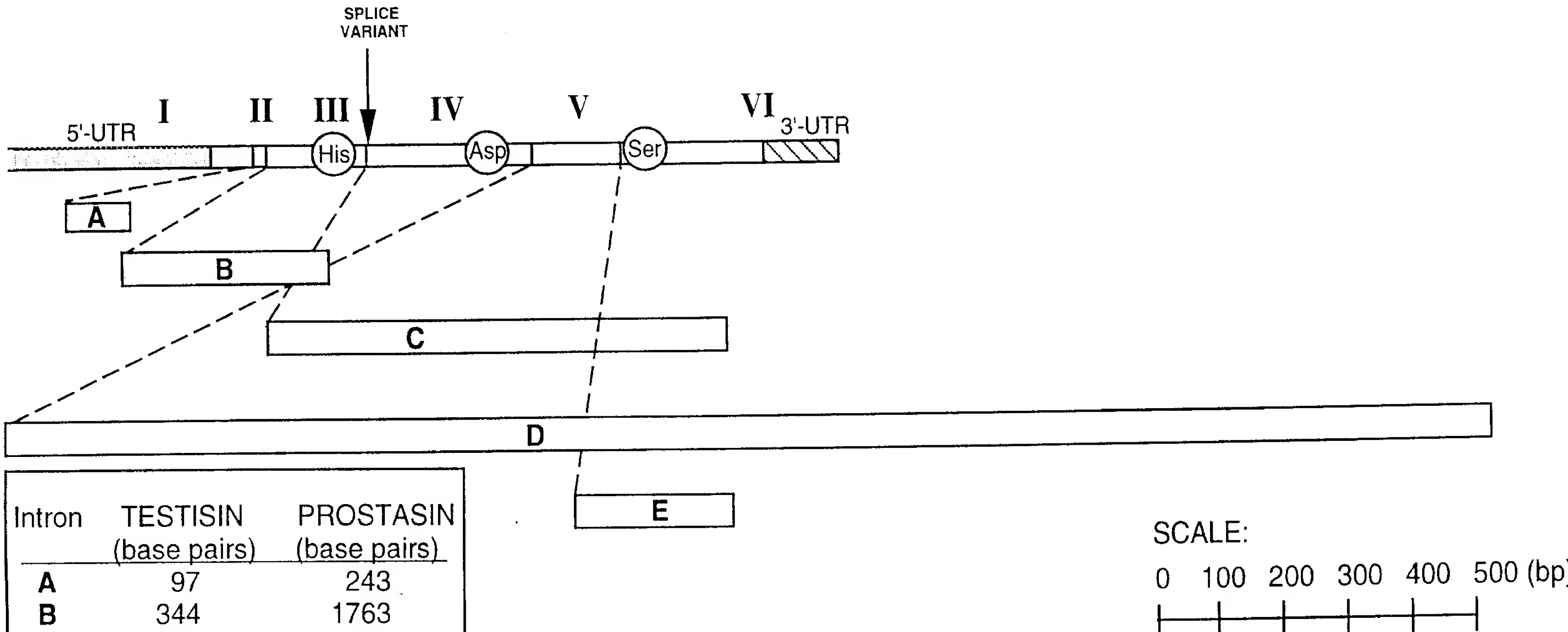
FIG 14

A. Northern Blot

B. Immunohistochemistry



TESTISIN INTRON/EXON BOUNDARIES AND SIZES

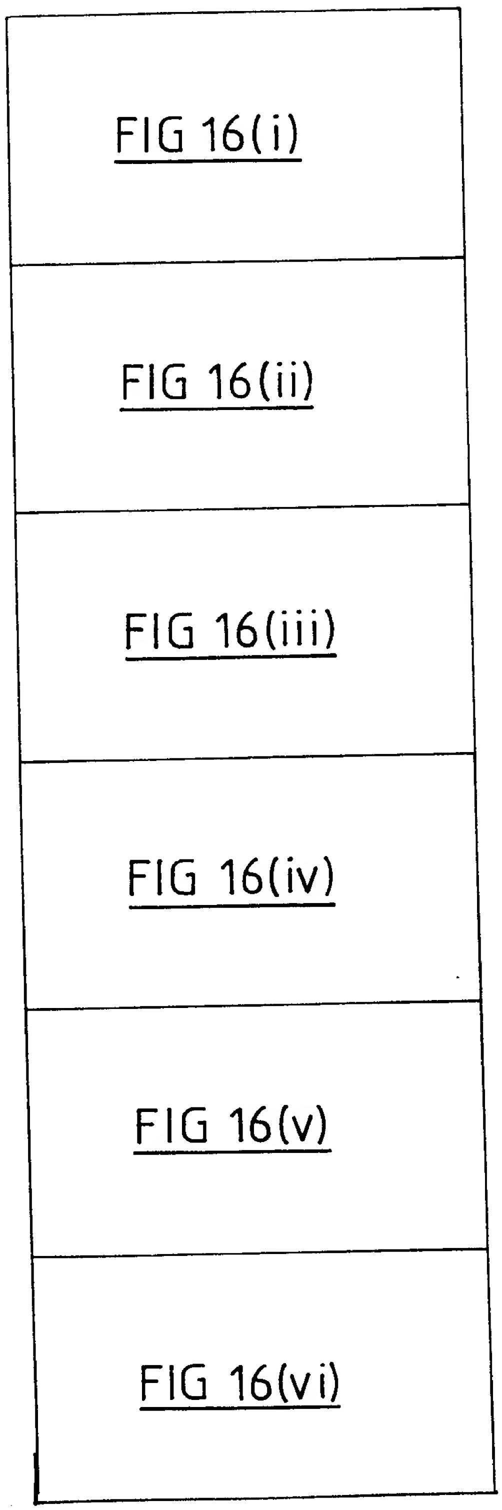


Intron	TESTISIN (base pairs)	PROSTASIN (base pairs)
A	97	243
B	344	1763
C	716	271
D	~2200	85
E	256	92

Exon	TESTISIN (base pairs)	PROSTASIN (base pairs)
I	>76	417
II	18	18
III	163	163
IV	284	272
V	168	167
VI	348	899

FIGURE 15

FIG 16



```
agtgagtctc ctgcctcagc ctcccaagta gctgggactt caggtgtgtg    50
ccaccatcct cagctaattt tttttttttt tttttttttg agaaggagtc   100
ttgctctgtc gcccaggctg gagtgcagtg gcgcgatctt ccaggcccca   150
ccgggccctc aggaaggcct tgcctacctg ctttaagggg actcctggct   200
cagggccagg cccctggtgc tggaggaggt ggtgggtgga gggcaggggg   250
caccaagcgg gcagccagga cccccgggct gcagacaaga aaaggactgt   300
                      /+1...EXON 1...
ggggtccacc gggtctgggc cACATCAAGG AATGTGGTTG AAGACCCGCC   350
CTTAGGAGCT GAAAGCCAGG GCGCTACCAG GCCTGAGAGG CCCCAAACAG   400
CCCTTGGGCC TGGTTTGGGA GGATTAAGCT GGAGCTCCCA ACCCGCCCTG   450
CCCCCAGGGG GCGACCCCGG GCCCGGCGCG AGAGGAGGCA GAGGGGGCGT   500
CAGGCCGCGG GAGAGGAGGC CATGGGCGCG CGCGGGGCGC TGCTGCTGGC   550
                                     /INTRON A...
GCTGCTGCTG GCTCGGGCTG GACTCAGGAA GCCGGgtgag ctcggggcgc   600
tgctggcggg atggggaggc gggggagcgg tggggaggac gggaggtgga   650
```

FIG 16(i)

```
                                          /EXON 2...
ggccgcgggg agtcacttct tgtctcccgc agAGTCGCAG GAGGCGGCGC   700
         /INTRON B...
CGTTATCAGg tagggcgccc aggacgcgcg attcctgcca gggccgttgg   750
gccgaggtgg acgggggcg gtgaggggt agagggggc ctttactgct   800
ctctcgcccc cgcccccggg atcgagaact ctgttggcgt ggaaagtaac   850
taacggacgc tggaggggga tgggcgggcc ctgcagagca cgtgggagga   900
tctccagtgt cacctacttc ctgctgcaca cacgcgaggg gaccctgggt   950
gggcaaaaac gtgctttccc ggacggggtt gaaggggaga aagggagagg  1000
tcgggcttgg ggggctgcct cccgcggctc agcagttcct ctgaccatcc  1050
     /EXON 3...
gagGACCATG CGGCCGACGG GTCATCACGT CGCGCATCGT GGGTGGAGAG  1100
GACGCCGAAC TCGGGCGTTG GCCGTGGCAG GGGAGCCTGC GCCTGTGGGA  1150
TTCCCACGTA TGCGGAGTGA GCCTGCTCAG CCACCGCTGG GCACTCACGG  1200
```

FIG 16(ii)

/INTRON C...

```
CGGCGCACTG CTTTGAAACg tgagtggggg tgcgaacgga ggggtgcggg   1250
gacgggcagg aacagggctg gagggagtgc caccgaactt tacctctggt   1300
ctgatgccag acttgggcgt gaaagttgtg cgtggatgcg gcctggtgtt   1350
ctcctgagcc ccaggctgtg ctgcagccgg ttacacccac tccagttccc   1400
tttgggtctc ctggagggaa ccctgttcag gttattccag aatgttcttc   1450
cagaacattt ccacacactt ttgggtattc tctccctttt tctttcaacc   1500
caaagttcac cactgaccat cccaccctca tccccctcc tggtggacgg   1550
tgcggtacag tgtggggcac tgagccaagg ccagcacccc cgggccgctg   1600
tgtggactcc atcctgccaa tcccacattg gcgtggtgca tctccccatt   1650
cctccttggg ctgcatgggg gtgcccctgg aggccttggc tcaatgcaag   1700
gctccttggg acagctctgg gaggtgacaa gaccccaccc ttctgctgca   1750
ggagcaggtc ctaggacttt ggttgtggtc tgtctgggct ccttcatttc   1800
tgcaggggac cctgggtgtt agcaagtagc agcaacacca cagtttcccc   1850
tcctgcactg gaccccagtt gtgctcaggt agccagccct ccatccaggg   1900
```

FIG 16(iii)

/EXON 4...

ccccctgactg ctctcttctc ttctgccagc tatagTGACC TTAGTGATCC 1950
CTCCGGGTGG ATGGTCCAGT TTGGCCAGCT GACTTCCATG CCATCCTTCT 2000
GGAGCCTGCA GGCCTACTAC ACCCGTTACT TCGTATCGAA TATCTATCTG 2050
AGCCCTCGCT ACCTGGGGAA TTCACCCTAT GACATTGCCT TGGTGAAGCT 2100
GTCTGCACCT GTCACCTACA CTAAACACAT CCAGCCCATC TGTCTCCAGG 2150
CCTCCACATT TGAGTTTGAG AACCGGACAG ACTGCTGGGT GACTGGCTGG 2200

/INTRON D...

GGGTACATCA AAGAGGATGA GGgtgaggct ggggacaggc gggtcaggga 2250
ggaactgtct ttgttcacct gttcccctgc ataggcacaa tagccccctg 2300
cttggtctgg gggtgcaggc tatgcccctc ttgcttgcag tctctcctca 2350
cctgccaggg cagggaccaa acacccagtt ctctcccttc caggggctgt 2400
gggggccaga aggagagtgt gagagggagg ccagtttggc gcaagcctgt 2450
gggtggtgcg gtggtggagg ggttctggag ggcttggcga cataaacctc 2500
atacttggat ttattcctgc atctttccac ctcccccagt gctcaccaat 2550

FIG 16(iv)

```
gccccaggca tca..............approx 1000 bp...........       3563
ccaggttgcc ccttccccca aggtctggct ttggatgctt atgtgaacac      ≈3613
cgttttaagt tgccttggcc ccttcctcgg ttcctttttg gctgaggaat      ≈3663
ctctccatgg ctgcaggcag ggccattgtt gccattctac agatagggaa      ≈3713
agtgcggctg ggggagctct gacagctgtc cctccccggg gccttctgtg      ≈3763
atgctgctga gggcctctgt tgtgctgggg tctgggttgg agctgggggt      ≈3813
aatggagatg aacctgccag gcacagtggg tgccccaggg cccccacccc      ≈3863
cgcagcctat gccatccctc catagagggg cctcaggttg ctgtctctct      ≈3913
                        /EXON 5...
ccttcccact atcgtccgca cagCACTGCC ATCTCCCCAC ACCCTCCAGG      ≈3963
AAGTTCAGGT CGCCATCATA AACAACTCTA TGTGCAACCA CCTCTTCCTC      ≈4013
AAGTACAGTT TCCGCAAGGA CATCTTTGGA GACATGGTTT GTGCTGGCAA      ≈4063
                                /INTRON  E...
TGCCCAAGGC GGGAAGGATG CCTGCTTCgt gagtgtcctt gccaccactc      ≈4113
ccagcccagg aaagcatcct gtgtccctgt gccttatttg accctcatgc      ≈4163
caaccccggg aggtggagac tgttgcccca ctctgcagat gcagaaacgg      ≈4213
```

FIG 16(v)

```
aggcttggct gctgccaggg ggaggaggag gatgtgcacc cagtctaccc     ≈4263
agccccatag cccttcccac tctcagcccc tcccctgccc cactcactct     ≈4313
                                      /EXON 6...
gccccaggct gacctcagcc ccgctgctcc ccagGGTGAC TCAGGTGGAC     ≈4363
CCTTGGCCTG TAACAAGAAT GGACTGTGGT ATCAGATTGG AGTCGTGAGC     ≈4413
TGGGGAGTGG GCTGTGGTCG GCCCAATCGG CCCGGTGTCT ACACCAATAT     ≈4463
CAGCCACCAC TTTGAGTGGA TCCAGAAGCT GATGGCCCAG AGTGGCATGT     ≈4513
CCCAGCCAGA CCCCTCCTGG CCGCTACTCT TTTTCCCTCT TCTCTGGGCT     ≈4563
CTCCCACTCC TGGGGCCGGT CTGAGCCTAC CTGAGCCCAT GCAGCCTGGG     ≈4613
GCCACTGCCA AGTCAGGCCC TGGTTCTCTT CTGTCTTGTT TGGTAATAAA     ≈4663
CACATTCCAG TTGATGCCTT GCAGGGCATT CTTCAaaagc agtggcttca     ≈4713
tggacagctc attctctctt gtgcagacag cctgtctgtg cccctggctc     ≈4763
acacccacat ctgttctgca ccatagaacc atctggttat ttcgatcaga     ≈4813
aagagaattg tgtgttgccc aggctggtct tgaacgccta gggtgtctcg     ≈4863
atc                                                        ≈4866
```

FIG 16(vi)

EXON III CACTGCTTTGAAAC**gt**gagtgggggtgcgaacggag
gggtgcggggacgggcaggaacagggctggagggagtgccaccga
actttacctctggtctgatgccagacttgggcgtgaaagttgtgc
gtggatgcggcctggtgttctcctgagccccaggctgtgctgcag
ccggttacacccactccagttccctttgggtctcctggagggaac
cctgttcaggttattccagaatgttcttccagaacatttccacac
acttttgggtattctctcccttttctttcaacccaaagttcacc
actgaccatcccaccctcatccccctcctggtggacggtgcggt
acagtgtggggcactgagccaaggccagcacccccgggccgctgt

...............INTRON C (716 BP)..............

gtggactccatcctgccaatcccacattggcgtggtgcatctccc
cattcctccttgggctgcatggggtgcccctggaggccttggct
caatgcaaggctccttgggacagctctgggaggtgacaagacccc
acccttctgctgcaggagcaggtcctagactttggttgtggtctg
tctgggctccttcatttctgcaggggaccctgggtgttagcaagt
agcagcaacaccacagtttcccctcctgcactggaccccagttgt
gctcaggtagccagccctccatccagggcccctgactgctctctt
ctcttctgcc**ag**ctat**ag**TGACCTTAGTGATCCC EXON IV

ALTERNATIVE SPLICING EVENTS

SHORT ISOFORM

CACTGCTTTGAAACTGACCTTAGTGATCCC

H C F E T D L S D P

LONG ISOFORM

CACTGCTTTGAAAC**CTATAG**TGACCTTAGTGATCCC

H C F E T **Y** **S** D L S D P

FIGURE 17

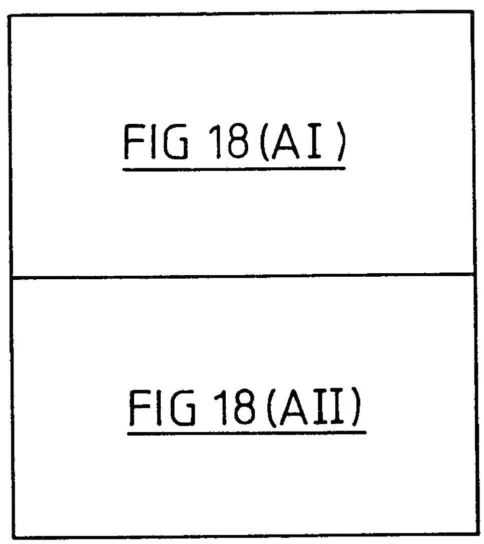


FIG 18(A)

FIGURE 18 (AI)

```
1    CGACCTATTGTCAGGGCCCTGCGGTCACAGGACCATCCCTTCCCGTATAGTGGGTGGCGA
      D  L  L  S  G  P  C  G  H  R  T  I  P  S  R  I  V  G  G  D   20

61   TGATGCTGAGCTTGGCCGCTGGCCGTGGCAAGGGAGCCTGCGTGTATGGGGCAACCACTT
      D  A  E  L  G  R  W  P  W  Q  G  S  L  R  V  W  G  N  H  L   40

121  ATGTGGCGCAACCTTGCTCAACCGCCGCTGGGTGCTTACAGCTGCCCACTGCTTCCAAAA
      C  G  A  T  L  L  N  R  R  W  V  L  T  A  A  H  C  F  Q  K   60

181  GGATAACGATCCTTTTGACTGGACAGTCCAGTTTGGTGAGCTGACTTCCAGGCCATCTCT
      D  N  D  P  F  D  W  T  V  Q  F  G  E  L  T  S  R  P  S  L   80

241  CTGGAACCTACAGGCCTATTCCAACCGTTACCAAATAGAAGATATTTTCCTGAGCCCCAA
      W  N  L  Q  A  Y  S  N  R  Y  Q  I  E  D  I  F  L  S  P  K  100

301  GTACTCGGAGCAGTATCCCAATGACATAGCCCTGCTGAAGCTGTCATCTCCAGTCACCTA
      Y  S  E  Q  Y  P  N  D  I  A  L  L  K  L  S  S  P  V  T  Y  120

361  CAATAACTTCATCCAGCCCATCTGCCTCCTGAACTCCACGTACAAGTTTGAGAACCGAAC
      N  N  F  I  Q  P  I  C  L  L  N  S  T  Y  K  F  E  N  R  T  140

421  TGACTGCTGGGTGACCGGCTGGGGGGCTATTGGAGAAGATGAGAGTCTGCCATCTCCCAA
      D  C  W  V  T  G  W  G  A  I  G  E  D  E  S  L  P  S  P  N  160
```

FIGURE 18 (AII)

```
481 CACTCTCCAGGAAGTGCAGGTAGCTATTATCAACAACAGCATGTGTAACCATATGTACAA
      T  L  Q  E  V  Q  V  A  I  I  N  N  S  M  C  N  H  M  Y  K 180

541 AAAGCCAGACTTCCGCACGAACATCTGGGGAGACATGGTTTGCGCTGGCACTCCTGAAGG
      K  P  D  F  R  T  N  I  W  G  D  M  V  C  A  G  T  P  E  G 200

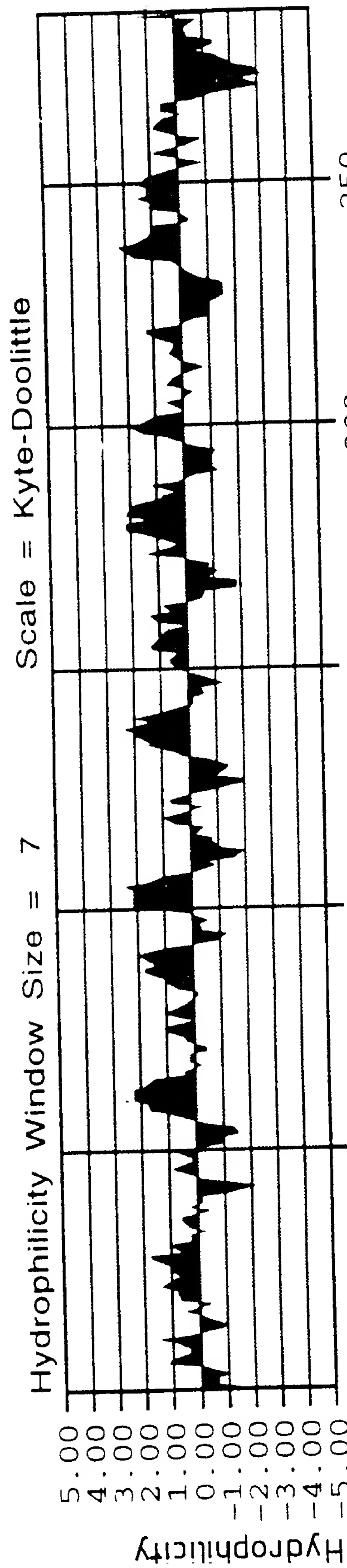
601 TGGCAAGGATGCCTGCTTTGGTGACTCGGGAGGACCCTTGGCCTGCGACCAGGATACGGT
      G  K  D  A  C  F  G  D  S  G  G  P  L  A  C  D  Q  D  T  V 220

661 GTGGTATCAGGTTGGAGTTGTGAGCTGGGGAATAGGCTGTGGTCGCCCCAATCGCCCTGG
      W  Y  Q  V  G  V  V  S  W  G  I  G  C  G  R  P  N  R  P  G 240

721 AGTCTATACCAACATCAGTCATCACTACAACTGGATCCAGTCAACCATGATCCGCAATGG
      V  Y  T  N  I  S  H  H  Y  N  W  I  Q  S  T  M  I  R  N  G 260

781 GCTGCTCAGGCCTGACCCAGTCCCCTTGCTACTGTTTCTTACTCTGGCCTGGGCTTCCTC
      L  L  R  P  D  P  V  P  L  L  L  F  L  T  L  A  W  A  S  S 280
841 TTTGCTGAGGCCTGCCTGAGCCCACACGTGTACGTCACACCTGTGAGGTCAGGGTGTGTC
      L  L  R  P  A                                              285

901 TCTTTTGTATCTTGCTTGCTAATAAACCTGTTAATATTTAAAAAAAAAAAAAAAAAAAA
```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50
100
150
200
250
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
Hydrophilicity

FIG 18B

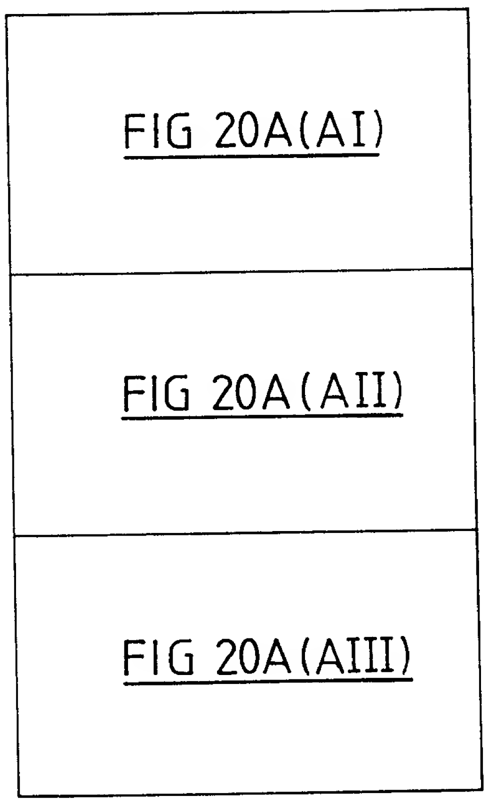


FIG 20A(A)

FIGURE 20A (AI)

```
    CTGAACCGGGTTGTGGGCGGCGAGGACAGCACTGACAGCGAGTGGCCCTGGATCGTGAGC  60
1    L  N  R  V  V  G  G  E  D  S  T  D  S  E  W  P  W  I  V  S

    ATCCAGAAGAATGGGACCCACCACTGCGCAGGTTCTCTGCTCACCAGCCGCTGGGTGATC 120
21   I  Q  K  N  G  T  H  H  C  A  G  S  L  L  T  S  R  W  V  I

    ACTGCTGCCCACTGTTTCAAGGACAACCTGAACAAACCATACCTGTTCTCTGTGCTGCTG 180
41   T  A  A  H  C  F  K  D  N  L  N  K  P  Y  L  F  S  V  L  L

    GGGGCCTGGCAGCTGGGGAACCCTGGCTCTCGGTCCCAGAAGGTGGGTGTTGCCTGGGTG 240
61   G  A  W  Q  L  G  N  P  G  S  R  S  Q  K  V  G  V  A  W  V

    GAGCCCCACCCTGTGTATTCCTGGAAGGAAGGTGCCTGTGCAGACATTGCCCTGGTGCGT 300
81   E  P  H  P  V  Y  S  W  K  E  G  A  C  A  D  I  A  L  V  R

    CTCGAGCGCTCCATACAGTTCTCAGAGCGGGTCCTGCCCATCTGCCTACCTGATGCCTCT 360
101  L  E  R  S  I  Q  F  S  E  R  V  L  P  I  C  L  P  D  A  S

    ATCCACCTCCCTCCAAACACCCACTGCTGGATCTCAGGCTGGGGGAGCATCCAAGATGGA 420
121  I  H  L  P  P  N  T  H  C  W  I  S  G  W  G  S  I  Q  D  G
```

FIGURE 20A (AII)

```
     GTTCCCTTGCCCCACCCTCAGACCCTGCAGAAGCTGAAGGTTCCTATCATCGACTCGGGAA 480
141   V  P  L  P  H  P  Q  T  L  Q  K  L  K  V  P  I  I  D  S  E

     GTCTGCAGCCATCTGTACTGGCGGGGAGCAGGACAGGGACCCATCACTGAGGACATGCTG  540
161   V  C  S  H  L  Y  W  R  G  A  G  Q  G  P  I  T  E  D  M  L

     TGTGCCGGCTAACTTGGAGGGGGAGCGGGATGCTTGTCTGGCGACTCCGGGGGCCCCCTC 600
181   C  A  G  Y  L  E  G  E  R  D  A  C  L  G  D  S  G  G  P  L

     ATGTGCCAGGTGGACGGCGCCTGGCTGCTGGCCGGCATCATCAGCTGGGGCGAGGGCTGT  660
201   M  C  Q  V  D  G  A  W  L  L  A  G  I  I  S  W  G  E  G  C

     GCCGAGCGCAACAGGCCCGGGGTCTACATCAGCCTCTCTGCGCACCGCTCCTGGGTGGAG  720
221   A  E  R  N  R  P  G  V  Y  I  S  L  S  A  H  R  S  W  V  E

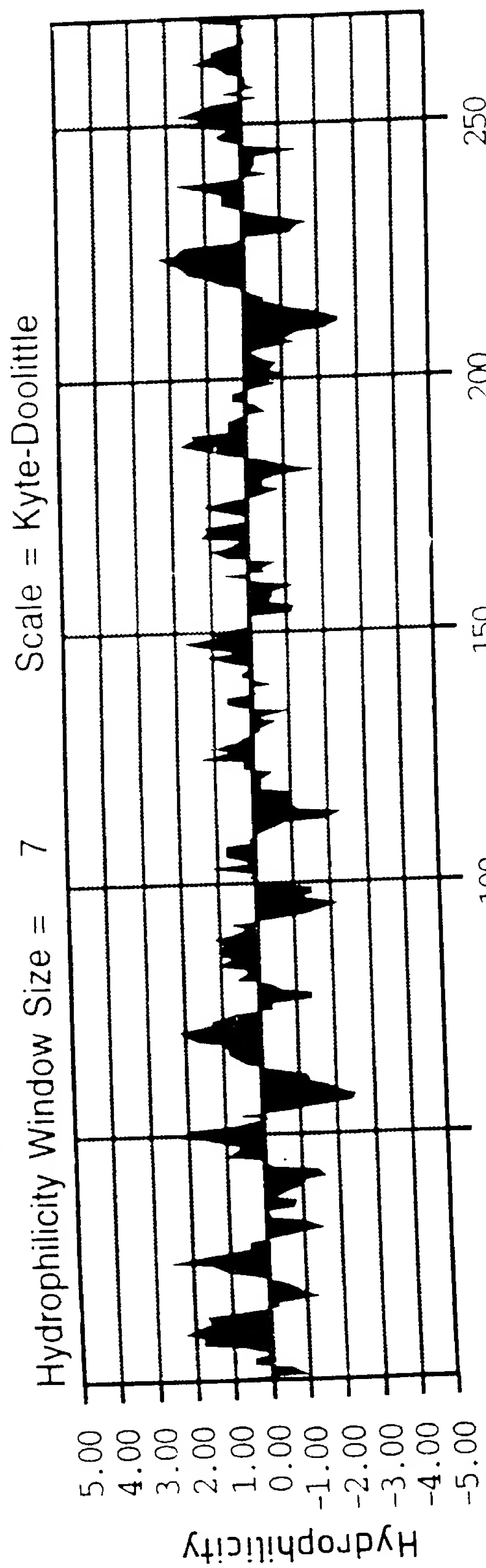
     AAGATCGTGCAAGGGGTGCAGCTCCGCGGCGCGCTCAGGGGGGTGGGCCCTCAGGGCA  780
241   K  I  V  Q  G  V  Q  L  R  G  R  A  Q  G  G  G  A  L  R  A

     CCGAGCCAGGGCTCTGGGGCCGCCGCGCGCTCCTAGGGGCCCAGCGGGACGCGGGGCTCGG 840
261   P  S  Q  G  S  G  A  A  A  R  S

     ATCTGAAAGGCGGCCAGATCCACATCTGGATCTGGATCTGCGGCGGCCTCGGGCGGTTTC  900
     CCCCGCCGTAAATAGGCTCATCTACCTCTACCTCTGGGGGCCCGGACGGCTGCTGCGGAA  960
```

FIGURE 20A (AIII)

```
AGGAAACCCCCTCCCCGACCCGCCCGACGGCCTCAGGCCCCGCCTCCAAGGCATCAGGCC 1020
CCGCCCAACGGCCTCATGTCCCCGCCCCCACGACTTCCGGCCCCGCCCCGGGCCCCAGCG 1080
CTTTTGTGTATATAAATGTTAATGATTTTTATAGGTATTTGTAACCCTGCCACATATCT  1140
TATTTATTCCTCCAATTTCAATAAA
```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Scale = Kyte-Doolittle
Hydrophilicity
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
50
100
150
200
250

FIG 20A (B)

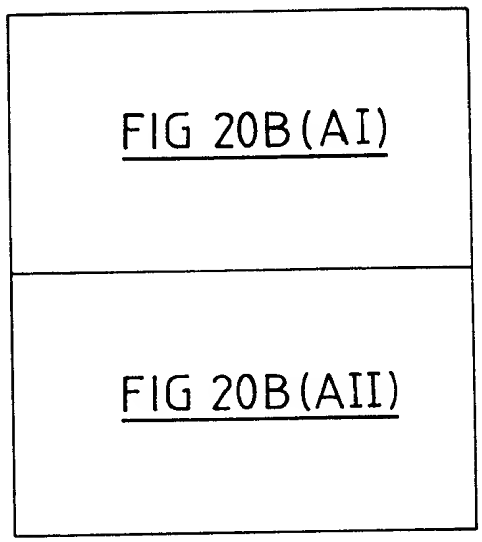


FIG 20B(A)

FIGURE 20B (AI)

```
     AATGCGGCCACTCCAAGGAGGCCGGGAGGATTGTGGGAGGCCAACACACCCAGGAAGGAC  60
1     C  G  H  S  K  E  A  G  R▽I  V  G  G  Q  D  T  Q  E  G

     GCTGGCCGTGGCAGGTTGGCCTGTGGTTGACCTCAGTGGGCATGTATGTGGGGCTCCC 120
21   R  W  P  W  Q  V  G  L  W  L  T  S  V  G  H  V  C  G  G  S

     TCATCCACCCACGCTGGGTGCTCACAGCCGCCCACTGCTTCCTGAGGTCTGAGGATCCCG 180
41   L  I  H  P  R  W  V  L  T  A  A  H  C  F  L  R  S  E  D  P

     GGCTCTACCATGTTAAAGTCGGAGGGCTGACACCCTCACTTTCAGAGCCCCACTCGGCCT 240
61   G  L  Y  H  V  K  V  G  G  L  T  P  S  L  S  E  P  H  S  A

     TGGTGGCTGTGAGGAGGCTCCTGGTCCACTCCTCATACCATGGACCACCACCAGCGGGG 300
81   L  V  A  V  R  R  L  L  V  H  S  S  Y  H  G  T  T  T  S  G

     ACATTGCCCTGATGGAGCTGGACTCCCCCTTGCAGGCCTCCCAGTTCAGCCCCATCTGCC 360
101  D  I  A  L  M  E  L  D  S  P  L  Q  A  S  Q  F  S  P  I  C

     TCCCAGGACCCCAGACCCCCCTCGCCATTGGGACCGTGTGCTGGGTAAACGGGCTGGGGG 420
121  L  P  G  P  Q  T  P  L  A  I  G  T  V  C  W  V  N  G  L  G

     TCCACTCAGGAGAGGCCCTGGCGAGTGTCCTTCAGGAGGTGGCTGTGCCCCTCCTGGACT 480
141  V  H  S  G  E  A  L  A  S  V  L  Q  E  V  A  V  P  L  L  D
```

FIGURE 20B (AII)

```
    CGAACATGTGTGAGCTGATGTACCACCTAGGAGAGCCCAGCCTGGCTGGCCAGCGCCTCA 540
161 S  N  M  C  E  L  M  Y  H  L  G  E  P  S  L  A  G  Q  R  L

    TCCAGGACGACATGCTCTGTGCTGGCTCTGTCCAGGGCAAGAAAGACTCCTGCCAGGGTG 600
181 I  Q  D  D  M  L  C  A  G  S  V  Q  G  K  K  D  S  C  Q  G

    ACTCCGGGGGGCCGCTGGTCTGCCCCATCAATGATACGTGGATCCAGGCCGGCATTGTGA 660
201 D  S  G  G  P  L  V  C  P  I  N  D  T  W  I  Q  A  G  I  V

    GCTGGGGATTCGGCTGTGCCCGGCCTTTCCGGCCTGGTGTCTACACCCAGGTGCTAAGCT 720
221 S  W  G  F  G  C  A  R  P  F  R  P  G  V  Y  T  Q  V  L  S

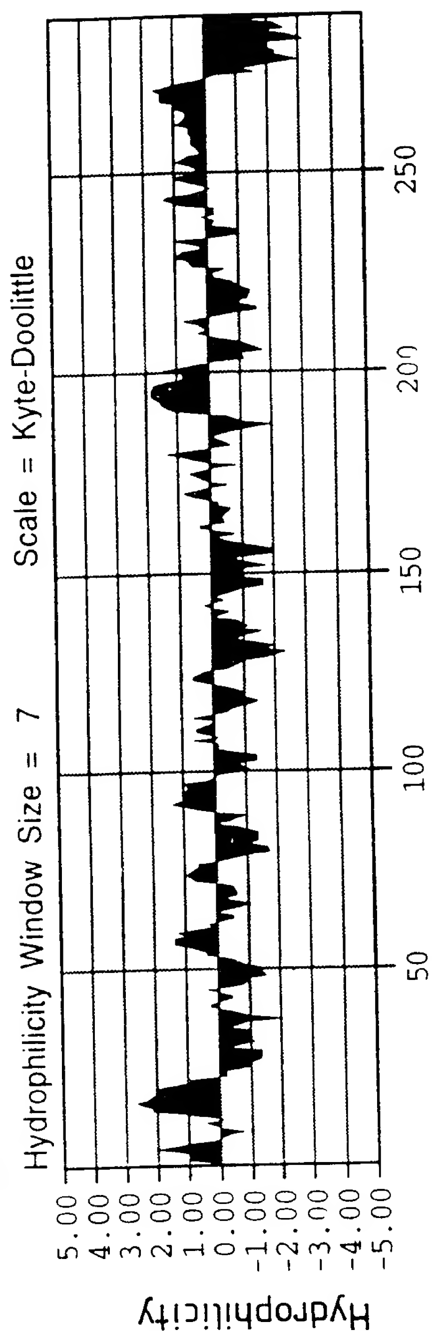
    ACACAGACTGGATTCAGAGAACCCTGGCTGAATCTCACTCAGGCATGTCTGGGGCCCGCC 780
241 Y  T  D  W  I  Q  R  T  L  A  E  S  H  S  G  M  S  G  A  R

    CAGGTGCCCCAGGATCCCACTCAGGCACCTCCAGATCCCACCCAGTGCTGCTGCTTGAGC 840
261 P  G  A  P  G  S  H  S  G  T  S  R  S  H  P  V  L  L  L  E

    TGTTGACCGTATGCTTGCTTGGGTCCCTGTGAACCATGAGCCATGGAGTCCGGGATCCCC 900
281 L  L  T  V  C  L  L  G  S  L

    TTTCTGGTAGGATTGATGGAATCTAATAATAAA
```

FIG 20B(B)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Scale = Kyte-Doolittle
Hydrophilicity
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
50
100
150
200
250

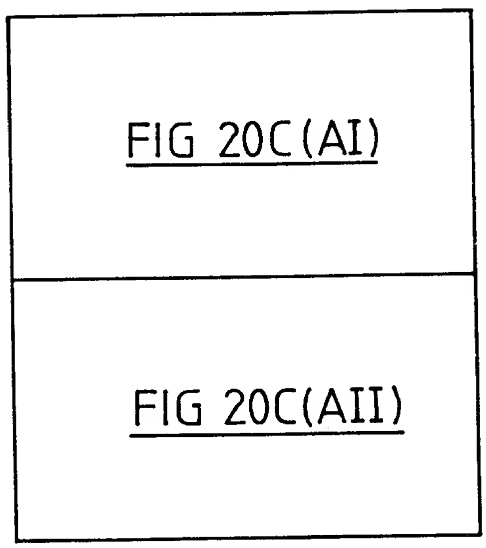


FIG 20C(A)

FIGURE 20C (AI)

```
        CCTGTGGTCGCCCCAGGATGCTGAACCGAATGGTGGGCGGGCAGGACACGCAGGAGGGCG  60
1         C  G  R  P  R  M  L  N  R▽M  V  G  G  Q  D  T  Q  E  G

        AGTGGCCCTGGCAAGTCAGCATCCAGCGCAACGGAAGCCACTTCTGCGGGGGCAGCCTCA 120
21      E  W  P  W  Q  V  S  I  Q  R  N  G  S  H  F  C  G  G  S  L

        TCGCGGAGCAGTGGGTCCTGACGGCTGCGCACTGCTTCCGCAACACCTCTGAGACGTCCC 180
41      I  A  E  Q  W  V  L  T  A  A  H  C  F  R  N  T  S  E  T  S

        TGTACCAGGTCCTGCTGGGGGCAAGGCAGCTAGTGCAGCCGGGACCACACGCTATGTATG 240
61      L  Y  Q  V  L  L  G  A  R  Q  L  V  Q  P  G  P  H  A  M  Y

        CCCGGGTGAGGCAGGTGGAGAGCAACCCCCTGTACCAGGGCACGGCCTCCAGCGCTGACG 300
81      A  R  V  R  Q  V  E  S  N  P  L  Y  Q  G  T  A  S  S  A  D

        TGGCCCTGGTGGAGCTGGAGGCACCAGTGCCCTTCACCAATTACATCCTCCCCGTGTGCC 360
101     V  A  L  V  E  L  E  A  P  V  P  F  T  N  Y  I  L  P  V  C

        TGCCTGACCCCTCGGTGATCTTTGAGACGGGCATGAACTGCTGGGTCACTGGCTGGGGCA 420
121     L  P  D  P  S  V  I  F  E  T  G  M  N  C  W  V  T  G  W  G

        GCCCCAGTGAGGAAGACCTCCTGCCCGAACCGCGGATCCTGCAGAAACTCGCTGTGCCCA 480
141     S  P  S  E  E  D  L  L  P  E  P  R  I  L  Q  K  L  A  V  P
```

FIGURE 20C (AII)

```
    TCATCGACACACCCAAGTGCAACCTGCTCTACAGCAAAGACACCGAGTTTGGCTACCAAC 540
161 I  I  D  T  P  K  C  N  L  L  Y  S  K  D  T  E  F  G  Y  Q

    CCAAAACCATCAAGAATGACATGCTGTGCGCCGGCTTCGAGGAGGGCAAGAAGGATGCCT 600
181 P  K  T  I  K  N  D  M  L  C  A  G  F  E  E  G  K  K  D  A

    GCAAGGGCGACTCGGGCGGCCCCCTGGTGTGCCTCGTGGGTCAGTCGTGGCTGCAGGCGG 660
201 C  K  G  D  S  G  G  P  L  V  C  L  V  G  Q  S  W  L  Q  A

    GGGTGATCAGCTGGGGTGAGGGCTGTGCCCGCCAGAACCGCCCAGGTGTCTACATCCGTG 720
221 G  V  I  S  W  G  E  G  C  A  R  Q  N  R  P  G  V  Y  I  R

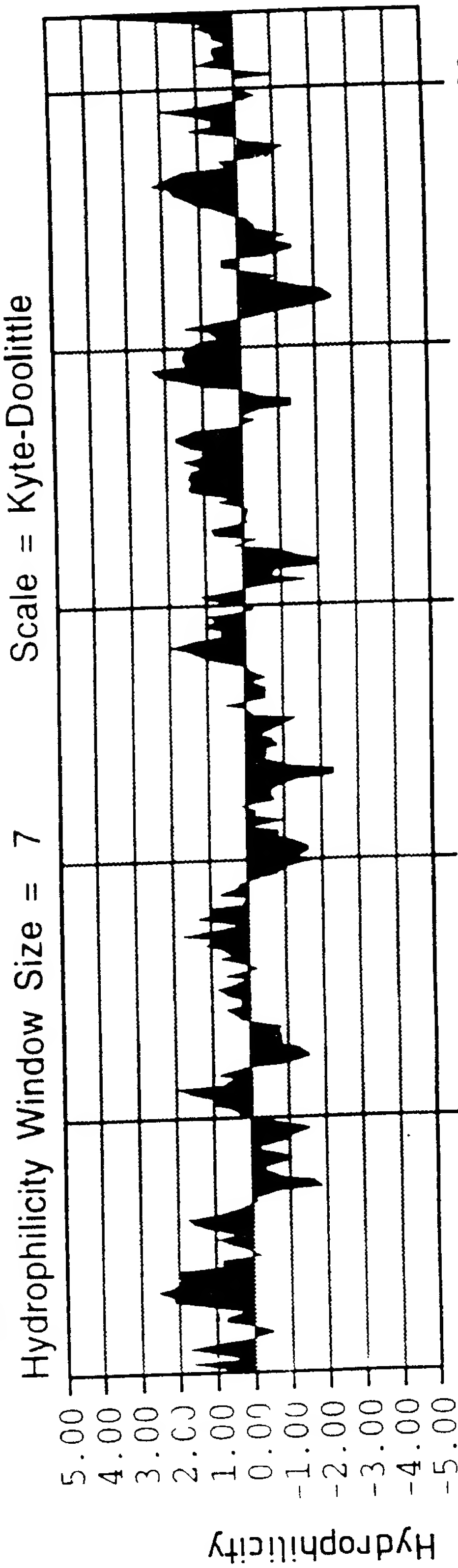
    TCACCGCCCACCACAACTGGATCCATCGGATCATCCCCAAACTGCAGTTCCAGCCAGCGA 780
241 V  T  A  H  H  N  W  I  H  R  I  I  P  K  L  Q  F  Q  P  A

    GGTTGGGCGGCCAGAAGTGAGACCCCCGGGGCCAGGAGCCCCTTGAGCAGAGCTCTGCAC 840
261 R  L  G  G  Q  K  *  D  P  R  G  Q  E  P  L  E  Q  S  S  A

    CCAGCCTGCCCGCCCACACCATCCTGCTGGTCCTCCCAGCGCTGCTGTTGCACCTGTGAG 900
281 P  S  L  P  A  H  T  I  L  L  V  L  P  A  L  L  L  H  L

    CCCCACCAGACTCATTTGTAAATAGCGCTCCTTCCTCCCCTCTCAAATACCCTTATTTTA 960
    TTTATGTTTCTCCCAATAAA
```

FIG 20C(B)


Hydrophilicity Window Size = 7 Scale = Kyte-Doolittle
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
Hydrophilicity
50
100
150
200
250

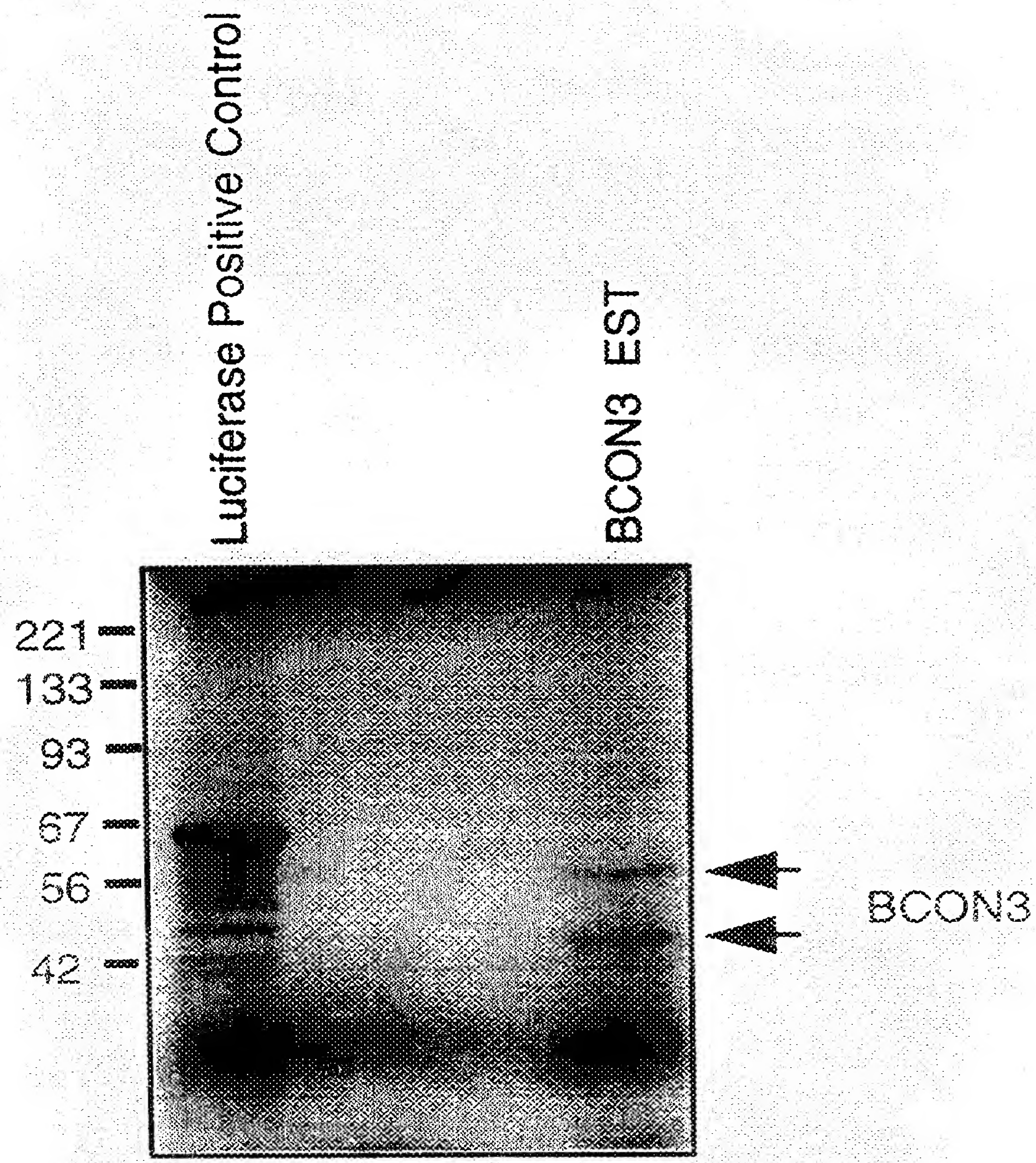

Luciferase Positive Control
BCON3 EST
221
133
93
67
56
42
BCON3

FIG 21

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00085

A.	**CLASSIFICATION OF SUBJECT MATTER**
Int Cl[6]:	C12N 009/12, 009/64, 015/54, 015/57; C07K 016/40; A61K 038/45, 038/48; C12Q 001/68

According to International Patent Classification (IPC) or to both national classification and IPC

B.	**FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
STN (DGENE) (see below)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEQUENCE DATABASES (see below) MEDLINE (see below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
(as online, STN (DGENE): TGGG[AC] [AGT] [GC] T [AGT] AC [AG] GC [AGT] GC [AGT] CA [CT] TG AND GG [AGT] CA [CT] [AT] [GC] [ACT] GG [ACT] CC [ACT] [CT] T and SWISSPROT, GENBANK, EMBL, PIR: SEQ ID Nos: 6, 8 and 10
MEDLINE: 16p13.3 AND "serine protease"

C.	**DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proc. Natl. Acad. Sci. USA 87, pages 960-3 (1990) Hanson, R.D. et al. "A cluster of hematopoetic serine protease genes is found on the same chromosomal band as the human α/S T-cell receptor locus." See whole document, especially page 961 column 2-962 column 1.	1,4,7,10,13,16,19,26, 27,31,32,35,38,46,49, 52,56,57,60,63

☒ Further documents are listed in the continuation of Box C

☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search 31 March 1998	Date of mailing of the international search report 03 APR 1998
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer **JIM CHAN** Telephone No.: (02) 6283 2340

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 19, 20, 26, 31, 44
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   the breadth of the claims was such that it was uneconomical to conduct a search that encompassed the full scope of the claims.

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid,. specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

C (Continuation)	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proc. Natl. Acad. Sci. USA 87, pages 3811-5 (1990) Vanderslice, P. et al. "Human mast cell tryptase: multiple cDNAs and genes reveal a multigene serine protease family." See whole document	1-4,5-7,10-13,14-16,26-28,31,32-38,44,46-52,55-59,60-63
X	J. Reprod. Fertil. 100, pages 567-75 (1994) Bermudez, D. et al. "Proacrosin as a marker of meiotic and post-meiotic germ cell differentiation: quantitative assessment of human spermatogenesis with a monoclonal antibody." See whole document, in particular Introduction.	46-48, 50, 51, 55
X	J. Biol. Chem. 269(29) pages 18843-8 (1994) Yu, J.X. et al. "Prostasin is a novel human serine protease from seminal fluid." See whole document, in particular discussion.	1-3,5,6,10-12,14,15,27,28,31-34,37,44,46-48,50,51,55-59,61,62
X	J. Biol. Chem. 269 (31) pages 19976-82 (1994) Matsushima, M. et al. "Structural characterisation of porcine enteropeptidase." See whole document, especially figure 4.	1-3,5,6,10-12,14,15,27,28,31-34,37,44,46-48,50,51,55-59,61,62
X	J. Biol. Chem. 270 (22) pages 13483-89 (1995) Yu, J.X. et al. "Molecular cloning, tissue-specific expression, and cellular localisation of human prostasin mRNA." See whole document, especially figure 2, Introduction and Discussion.	1-3,5,6,10-12,14,15,27,28,31-34,36,37,41-43,44,46-48,50,51,55-59,61,62
X	Mol. Reprod. Dev. 43, pages 236-47 (1996) O'Brien, D.A. et al. "Boar proacrosin" expressed in spermatids of transgenic mice does not reach the acrosome and disrupts spermatogenesis." See whole document.	1-3,5,6,10-12,14,15,27,28,31-34,36,37,41-43,44,46-48,50,51,55-59,61,62